PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 12 October 2000 (12.10.00)	
International application No. PCT/US00/04642	**Applicant's or agent's file reference** 3037.86704
International filing date (day/month/year) 24 February 2000 (24.02.00)	**Priority date** (day/month/year) 24 February 1999 (24.02.99)
Applicant ADAMS, Lynn, M. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

18 September 2000 (18.09.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Juan Cruz
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the RECEIVING OFFICE

To:
SARAH A. KAGAN
BANNER & WITCOFF, LTD
1001 G STREET, N.W.
ELEVENTH FLOOR
WASHINGTON, DC 20001 4597

PCT

NOTIFICATION OF THE INTERNATIONAL APPLICATION NUMBER AND OF THE INTERNATIONAL FILING DATE

(PCT Rule 20.5(c))

Date of mailing *(day/month/year)*	28 MAR 00
Applicant's or agent's file reference: 3037.86704	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/04642	24 FEB 00	24 FEB 99

Applicant
CASE WESTERN RESERVE UNIVERSITY

Title of the invention
ENHANCERS OF CFTR CHLORIDE CHANNEL FUNCTION

1. The applicant is hereby notified that the international application has been accorded the international application number and the international filing date indicated above.

2. The applicant is further notified that the record copy of the international application:

☒ was transmitted to the International Bureau on 28 MAR 00.

☐ has not yet been transmitted to the International Bureau for the reason indicated below and a copy of this notification has been sent to the International Bureau*:

☐ because the necessary national security clearance has not yet been obtained.

☐ because *(reason to be specified)*:

* The International Bureau monitors the transmittal of the record copy by the receiving Office and will notify the applicant (with Form PCT/IB/301) of its receipt. Should the record copy not have been received by the expiration of 14 months from the priority date, the International Bureau will notify the applicant (Rule 22.1(c)).

3. FOREIGN TRANSMITTAL LICENSE INFORMATION Completed by: L & R

☐ Additional license for foreign transmittal not required. This subject matter is covered by a license already granted or the equivalent U.S. national application. Refer to that license for information concerning its scope.

☐ License for foreign transmittal not required. 37 CFR. 5.11(e)(1) or 37 CFR 5.11(e)(2). However, a license may be required for additional subject matter. See 37 CFR 5.15(b).

☒ Foreign transmittal license granted. 35 U.S.C. 184; 37 CFR 5.11 on 15MAR2000 (date):

☒ 37 CFR 5.15(a) ☐ 37 CFR 5.15(b)

Name and mailing address of the receiving Office Assistant Commissioner for Patent Box PCT Washington, D.C. 20231 Attn:RO/US Facsimile No. 703-305-3230	Authorized officer Paul F. Urrutia Telephone No. 703-305-3681

Form PCT/RO/105 (July 1992)

PATENT COOPERATION TREATY

PCT

From the RECEIVING OFFICE

To:
SARAH A. KAGAN
BANNER & WITCOFF, LTD
1001 G STREET, N.W.
ELEVENTH FLOOR
WASHINGTON, DC 20001 4597

INVITATION TO CORRECT DEFECTS IN THE INTERNATIONAL APPLICATION

(PCT Articles 3(4)(i) and 14(1) and Rule 26)

Date of mailing *(day/month/year)*	28 MAR 00
REPLY DUE	within 1 months / days from the above date of mailing
Applicant's or agent's file reference: 3037.86704	International filing date *(day/month/year)*: 24 FEB 00
International application No.: PCT/US00/04642	
Applicant: CASE WESTERN RESERVE UNIVERSITY	

1. The applicant is hereby **invited**, within the time limit indicated above, to correct the defects **in the international application as filed**, the defects specified on the attached
   - [X] Annex A
   - [X] Annex B1 *(text matter of the international application as filed)*
   - [X] Annex C1 *(drawings of the international application as filed)*

2. The applicant is hereby **invited**, within the time limit indicated above, to correct the defects **in the translation of the international application** furnished under Rule 12.3, the defects specified on the attached
   - [ ] Annex A
   - [ ] Annex B2 *(text matter of the translation of the international application)*
   - [ ] Annex C2 *(drawings of the translation of the international application)*

Additional observations (if necessary):

The indication of "continuation" or "continuation-in-part" is not made next to the designation of United States of America in Box No. V of the international application identified above. However, the required information for the prior application (i.e., country of filing, application number and filing date) is indicated in the Supplemental Box of the Request form. A proposed replacement sheet containing the required information should be submitted. See PCT Rule 4.14 and the PCT Applicant's Guide, Volume I/A, paragraph 90.

HOW TO CORRECT THE DEFECTS?

Correction must be submitted by filing a replacement sheet embodying the correction and a letter accompanying the replacement sheet, which shall draw attention to the difference between the replaced sheet and the replacement sheet. A correction may be stated in a letter only if it is of such a nature that it can be transferred from the letter to the record copy without adversely affecting the clarity and direct reproducibility of the sheet onto which the correction is to be transferred (Rule 26.4(a)).

ATTENTION

Failure to correct the defects will result in the international application being considered withdrawn by this receiving Office (see Rule 26.5 for further details).

A copy of this invitation and any attachments has been sent to the International Bureau and the International Searching Authority.

Name and mailing address of the receiving Office Assistant Commissioner for Patent Box PCT Washington, D.C. 20231 Attn:RO/US Facsimile No. 703-305-3230	Authorized officer Paul F. Urrutia Telephone No. 703-305-3681

Form PCT/RO/106 (July 1998)

ANNEX A FORM PCT/RO/106

International application No.
PCT/US00/04642

The receiving Office has found the following defects in the international application as filed:

1. As to **signature*** of the international application (Rules 4.15 and 90.4), the request:
   a. ☐ is not signed.
   b. ☐ is not signed by all applicants.
   c. ☐ is not accompanied by the statement referred to in the check list in Box No. VIII of the request explaining the lack of the signature of an applicant for the designation of the United States of America.
   d. ☒ is signed by what appears to be an agent/common representative but
      ☒ the international application is not accompanied by a power of attorney appointing him.
      ☐ the power of attorney accompanying the international application was not signed by all the applicants.
   e. ☐ other (*specify*):

* All applicants must sign, including inventors if they are also applicants (e.g. where the United States of America is designated).

2. As to indications concerning the **applicant**, the request (Rules 4.4 and 4.5):
   a. ☐ does not properly indicate the applicant's name (*specify*):
   b. ☐ does not indicate the applicant's address.
   c. ☐ does not properly indicate the applicant's address (*specify*):
   d. ☐ does not indicate the applicant's nationality.
   e. ☐ does not indicate the applicant's residence.
   f. ☐ other (*specify*):

3. As to the **language** of certain elements of the international application, other than the description and claims (Rules 12.1(c) and 26.3*ter(a) and (c)*):
   a. ☐ the **request** is not in a language which is both a language accepted by this receiving Office and a language of publication, which is (are):
   b. ☐ the **text matter of the drawings** is not in the language in which the international application is to be published, which is:
   c. ☐ the **abstract** is not in the language in which the international application is to be published, which is:

4. The **title** of the invention:
   a. ☐ is not indicated in Box No. I of the request (Rule 4.1(a)).
   b. ☐ is not indicated at the top of the first sheet of the description (Rule 5.1(a)).
   c. ☐ as appearing in Box No. I of the request is not identical with the title heading the description (Rule 5.1(a)).

5. As to the **abstract** (Rule 8):
   ☐ the international application does not contain an abstract.

ANNEX B FORM PCT/RO/106

International application No.
PCT/US00/04642

The receiving Office has found that, with regard to the presentation of the **text matter of the international application as filed**, the physical requirements are not complied with to the extent that compliance therewith is necessary for:

1. [X] reasonably uniform international publication (Rules 11 and 26.3(a)(i)) *(defects to be specified)*:

		Request	Description	Claims	Abstract
a. [ ]	The sheets do not admit of direct reproduction.	[ ]	[ ]	[ ]	[ ]
b. [ ]	The element does not commence on a new sheet.	[ ]	[ ]	[ ]	[ ]
c. [ ]	Sheets are not free from creases, cracks, folds.	[ ]	[ ]	[ ]	[ ]
d. [ ]	Sheets are not used in the upright position.	[ ]	[ ]	[ ]	[ ]
e. [ ]	One side of the sheets is not left unused.	[ ]	[ ]	[ ]	[ ]
f. [ ]	The paper of the sheets is not flexible/strong/white/smooth/non-shiny/durable.	[ ]	[ ]	[ ]	[ ]
g. [ ]	The sheets are not connected as prescribed (Rule 11.4(b)).	[ ]	[ ]	[ ]	[ ]
h. [ ]	Sheets are not A4 size (29.7cm x 21cm).	[ ]	[ ]	[ ]	[ ]
i. [X]	The minimum margins on the sheets are not as prescribed (top: 2cm; left side: 2.5cm; right side: 2cm; bottom: 2cm).		[X]	[X]	[X]
j. [ ]	The file reference number indicated on the sheets does not appear in the left-hand corner of the sheets, within 1.5cm of the top of the sheets.		[ ]	[ ]	[ ]
k. [ ]	The file reference number exceeds the maximum of 12 characters.	[ ]	[ ]	[ ]	[ ]
l. [ ]	The sheets of the description, claims and abstract are not numbered in consecutive Arabic numerals.		[ ]	[ ]	[ ]
m. [ ]	The sheet numbers are not centered at the top or bottom of the sheets.	[ ]	[ ]	[ ]	[ ]
n. [ ]	The sheet numbers are in the margin (see i. above for the size of the margins).		[ ]	[ ]	[ ]
o. [ ]	The text matter is not typed or printed.	[ ]	[ ]	[ ]	[ ]
p. [ ]	The typing on the sheets is not 1.5-spaced.		[ ]	[ ]	[ ]
q. [ ]	The characters in the text matter on the sheets are less than 0.21 cm high in capital letters.	[ ]	[ ]	[ ]	[ ]
r. [ ]	The text matter on the sheets is not in dark, indelible color.	[ ]	[ ]	[ ]	[ ]
s. [ ]	The element contains drawings.	[ ]	[ ]	[ ]	[ ]
t. [ ]	The sheets contain alterations/overwritings/interlineations/too many erasures.	[ ]	[ ]	[ ]	[ ]
u. [ ]	The sheets contain photocopy marks.	[ ]	[ ]	[ ]	[ ]

2. [ ] satisfactory reproduction (Rules 11 and 26.3(b)(i)).

Further observation (if necessary):

ALL NEW SHEETS REQUIRED

ANNEX C FORM PCT/RO/105

International application No.
PCT/US00/04642

The receiving Office has found that, with regard to the presentation of the drawings of the international application as filed, the physical requirements are not complied with to the extent that compliance therewith is necessary for:

1. [X] reasonally uniform international publication (Rules 11 and 26.3(a)(i)) *(defects to be specified)*:

Sheets containing drawings:

a. [ ] the sheets do not admit of direct reproduction.
b. [ ] the sheets are not free from creases, cracks, folds.
c. [ ] one side of the sheets is not left unused.
d. [ ] the paper of the sheets is not flexible/strong/white/smooth/non-shiny/durable.
e. [ ] the drawings do not commence on a new sheet.
f. [ ] the sheets are not connected as prescribed (Rule 11.4(b)).
g. [ ] the sheets are not A4 size (29.7cm x 21cm).
h. [X] the minimum margins on the sheets are not as prescribed (top: 2.5cm; left side: 2.5cm; right side: 1.5cm; bottom: 1cm).
i. [ ] the file reference number indicated on the sheets does not appear in the left-hand corner of the sheets, within 1.5cm of the top of the sheets.
j. [ ] the file reference number exceeds the maximum of 12 characters.
k. [ ] the sheets are not free from frames around usable or used surfacts.
l. [X] the sheets are not numbered in consecutive Arabic numerals (e.g. 1/3, 2/3, 3/3).
m. [ ] the sheet numbers are not centered at the top or bottom of the sheets.
n. [ ] the sheet numbers are in the margin (see h. above for the size of the margins).
o. [ ] the sheets contain alterations/overwritings/interlineations/too many erasures.
p. [ ] the sheets contain photocopy marks.

Drawings (Rule 11.13):

a. [X] do not admit of direct reproduction.
b. [ ] contain unnecessary text matter.
c. [ ] contain words so placed as to prevent translation without interference with lines thereof.
d. [X] are not executed in durable black color; the lines are not uniformly thick and well-defined.
e. [ ] contain cross-sections not properly hatched.
f. [ ] would not be properly distinguishable in reduced reproduction.
g. [ ] contain scales not represented graphically.
h. [ ] contain numbers, letters and reference lines lacking simplicity and clarity.
i. [ ] contain lines drafted without the aid of drafting instruments.
j. [ ] contain disproportionate elements of a figure not necessary for clarity.
k. [ ] contain numbers and letters of height less than 0.32 cm.
l. [ ] contain letters not conforming to the Latin, and where customary, Greek alphabets.
m. [ ] contain figures on two or more sheets which form a single complete figure but which are not able to be assembled without concealing parts thereof.
n. [ ] contain figures which are not properly arranged and clearly separated.
o. [ ] contain different figures not numbered in consecutive Arabic numerals.
p. [ ] contain different figures not numbered independent of the numbering of the sheets.
q. [ ] are not restricted to reference signs mentioned in the description.
r. [ ] do not contain reference signs that are mentioned in the description.
s. [ ] contain the same feature denoted by different reference signs.
t. [ ] are not arranged in an upright position, clearly separated from one another.
u. [ ] are not presented sideways with the top of the figures at the left side of the sheets.

2. [X] satisfactory reproduction (Rules 11 and 26.3(b)(i)).

Further observations (if neccesary):

ALL NEW DRAWINGS REQUIRED

TO:
SARAH A. KAGAN
BANNER & WITCOFF, LTD
1001 G STREET, N.W.
ELEVENTH FLOOR
WASHINGTON, DC 20001 4597

UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

NOTIFICATION OF STATUS OF REQUIREMENTS UNDER 35 U.S.C. 371

DATE OF MAILING (day/month/year): 28 MAR 00

FILE REFERENCE: 3037.86704

IDENTIFICATION OF INTERNATIONAL APPLICATION

International application No.	International filing date (day/month/year)	Priority Date Claimed
PCT/US00/04642	24 FEB 00	24 FEB 99

Applicant for DO/EO/US: ADAMS, LYNN M.

NOTIFICATION

The applicant is hereby advised that the U.S. Patent and Trademark Office in its capacity as [X] Designated Office [ ] Elected Office has received following items as of the date of mailing indicated above.

1. [ ] U.S. Nation fee [35 U.S.C 371 (c) (1)]
2. [ ] Oath of declaration [35 U.S.C 371 (c) (4)]
3. [X] Copy of International application as [35 U.S.C 371 (c) (2)]
4. [ ] Translation of Application [35 U.S.C 371 (c) (2)]
5. [ ] Amendments under PCT Article 19 [35 U.S.C 371 (c) (3)]
6. [ ] Translation of PCT Article 19 Amendments [35 U.S.C 371 (c) (3)]
7. [ ] Search Report or Declaration under PCT Article 17(2) [35 U.S.C 371 (a)]
8. [ ] International Preliminary Examination Report and its Annexes, if any, under PCT Article 36(3)(b) [35 U.S.C 371 (a)]
9. [ ] Translation of Annexs to the International Preliminary Examination Report under PCT Article 36(3)(b) [35 U.S.C 371 (c) (5)]
10. [ ] Other items received:
    [ ] Assignment Document [ ] Prior Art Statement [ ] Preliminary Amendment

A. [ ] Requirements for U.S. National processing have been met. Processing will commence
   [ ] at the expiration of the applicable time limit under either
      [ ] PCT Article 22 [35 U.S.C 371 (b)] or
      [ ] PCT Article 39 [35 U.S.C 371 (b)]
   [ ] on the date indicated below under the provisions of 35 U.S.C 371 (f)

U.S. NATIONAL SERIAL#	DATE UNDER 35 U.S.C. 102(e)	DATE OF COMMENCEMENT OF NATIONAL PROCESSING

All correspondence submitted after the date of commencement of U.S. National processing indicated above should refer to the U.S. National Serial Number and the appropriate U.S. National processing organization of Officer.

B. [ ] As the above identified application has been accepted for U.S. National processing under the provision of 35 U.S.C.371 (f) before expiration of the applicable time limit under [ ] PCT Article 22 [ ] PCT Article 39, applicant is reminded that
   [ ] Amendments under PCT Article 19 and/or
   [ ] the International Preliminary Examination Report and its Annexes, if any, under PCT Article 36(3) (a), and (b) and any translation thereof, if applicable, must be submitted to the Patent and Trademark Office as soon as they are available.

International application No.	International filing date	Priority Date Claimed
PCT/US00/04642	24 FEB 00	24 FEB 99

C. [X] In order that U.S. National processing may begin, certain items must be received by the DO/EO/US by the expiration of applicable time limit under

- [X] PCT Article 22 or
- [X] PCT Article 39.

Specifically:

- [X] 1. U.S. National Fee
- [X] 2. Oath or Declaration
- [ ] 3. Copy of Application
- [ ] 4. Translation of application
- [X] 5. Amendments under PCT Article 19, if any
- [ ] 6. Translation of PCT Article 19 Amendments, if applicable
- [ ] 7. Search Report or PCT Article 17(2) declaration
- [ ] 8. International Preliminary Examination Report and its Annexes, if any, under PCT Article 36(3)(a), if applicable
- [ ] 9. Translation of Annexs to the International Preliminary Examination Report under PCT Article 36(3)(b), if appliable

THE ABOVE CHECK ITEMS MUST BE TIMELY RECEIVED TO AVOID ABANDONMENT OF THE APPLICATION. [35. U.S.C. 371(d)]

D. Further information for the applicant:

This is only a reminder.

UNITED STATES DESIGNATED/ELECTED OFFICE

Address Only:	Authorized Office
Assistant Commissioner for Patent Box PCT Washington, D.C. 20231 Attn:RO/US	Paul F. Urrutia

SAK
AMA

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:
BANNER & WITCOFF, LTD.
Attn. KAGAN, Sarah A.
1001 G Street, N.W.
Eleventh Floor
Washington, DC 20001-4597
UNITED STATES OF AMERICA

INVITATION TO FURNISH NUCLEOTIDE AND/OR AMINO ACID SEQUENCE LISTING COMPLYING WITH WIPO STANDARD ST25

(PCT Rule 13ter.1(a) and (c) and Administrative Instructions, Section 208 and Annex C)

Applicant's or agent's file reference 3037.86704	Date of mailing (day/month/year) 27/04/2000
International application No. PCT/US 00/04642	**REPLY DUE** within 1 months/~~days~~ from the above date of mailing
Applicant CASE WESTERN RESERVE UNIVERSITY et al.	International filing date (day/month/year) 24/02/2000

1. The applicant is hereby **invited**, within the time limit indicated above, to furnish to this Authority:

   [X] a nucleotide and/or amino acid sequence listing **in written form** complying with the standard provided for in Annex C of the Administrative Instructions, accompanied by a **statement** to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

   [ ] a **statement** to the effect that the sequence listing in written form, already furnished to this Authority, does not go beyond the disclosure in the international application as filed.

   [X] a nucleotide and/or amino acid sequence listing **in computer readable form** complying with the standard provided for in Annex C of the Administrative Instructions, accompanied by a **statement** that the information recorded in computer readable form is identical to the written sequence listing.

   [ ] a **statement** that the information recorded in computer readable form (that computer readable form having already been furnished to this Authority) is identical to the written sequence listing.

2. **Failure to comply with this invitation** may result in this Authority not carrying out the international search to the extent that no meaningful search can be carried out.

3. Further observations (if necessary):

IMPORTANT REMARK
The statements are legally required [See Suppl No 2 to Official Journal No 11/1998 (page 14, ψ 37 & 40 and page 64 ψ III.2)]

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Mirella Deleye-Milani M. Deleye

Form PCT/ISA/225 (July 1998)

Office Européen des Brevets DG1
Europäisches PatentAmt DG1
European Patent Office DG1

Storage and Retrieval of Amino acid and Nucleotide Data

P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk
Ms Mirella DELEYE-MILANI Room : S 02 N 24
Tel : + 31 70 340 31 54 **Fax : + 31 70 340 39 92**

ANNEX

Dear applicant/representative,

Present application contains amino acid/nucleotide sequences. For patent purposes, fragments and derivatives of known sequences not described as such in the prior art should be included in the sequence listing.

According to Supplement 2 to the Official Journal Nr.11/98 of the EPO (& Rules 13 ter & 5.2. PCT), if nucleotide/amino acid sequences **are disclosed** in a European/International patent application, the description shall contain a sequence listing complying with WIPO ST.25.

Thank you for the filed sequence listing but this listing is not complying with WIPO ST 25. (please see documentation enclosed).

Moreover, the computer readable form is missing.

The ISA hereby invites the applicant to submit a sequence listing, with appropriate annotations for each sequence (where applicable), **both on paper and in computer readable form, accompanied by the appropriate statements.**

Relating to this, we remind you that if these requirements are not met or not met in due time, the EPO does not perform the international search where a meaningful search cannot be carried out (Rule 13ter.1(c)PCT). In this case the international search report is replaced in full or in part by the statement under Article 17(2)(a)(ii)PCT.

Moreover Rule 13ter(f)prescribes that a subsequently filed sequence listing, which is not a correction within the meaning of rule 26.4 PCT and which is not a rectification within the meaning of Rule 91.1.PCT of a sequence listing, shall not form part of the international application. In accordance herewith, the furnishing of a subsequently filed sequence listing does not give rise to an opportunity either to amend the description, claims and figures with a view to refer to said subsequently filed sequence listing or add it to the application as originally filed. The subsequently furnished listing will therefore normally not be forwarded to the international Bureau for publication purposes

Th computer readable form of th Sequence Listing in ASCII f rmat (text nly) is mandatory.
For further questions do not h sitate to contact us.

../...

To avoid any possible processing delay, please send sequence listing on paper and in computer-readable form preferably to the
European Patent Office, STRAND Program, Directorate Biotechnology (Dir 1212),
Attn. : Ms M. Deleye, Room S 02 N 24 -
Patentlaan 2, NL 2288 EE RIJSWIJK (NL)

REMARK:

We strongly recommend the applicant to use the PatentIn software to submit the sequence listing. (If problems arise with the download of the Patentin software, a CD-ROM copy can-be obtained trough Ms van Laar-Rabelink - , Tel : +31 70 340 44 40 ; Fax : +31 70 340 3956 ; E-mail : epoline@epo.org).

The new Patentin is available on our EPO website with following address
www.european-patent-office.org/filingsoft/strand
Download is performed from that site .
Please read carefully the information provided on that site.

The downloaded install.exe file can be used
for the installation of the new version from Patentin.
W_UPATIN.EXE is the file to start PatentIn

Would you encounter problems, please take contact
with our Helpdesk epoline@epo.org.

Del 0./SAK

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PATENT COOPERATION TREATY

RECEIVED

From the RECEIVING OFFICE

To:
SARAH A. KAGAN
BANNER & WITCOFF, LTD
1001 G STREET, N.W.
ELEVENTH FLOOR
WASHINGTON, DC 20001 4597

Dead
Due 9-24-00

PCT

AUG 17 2000

BANNER & WITCOFF LTD.

COMMUNICATION IN CASES FOR WHICH NO OTHER FORM IS APPLICABLE

	Date of mailing *(day/month/year)* 14 AUG 2000
Applicant's or agent's file reference 3037.86704	REPLY DUE See paragraph 1 below
International application No. PCT/US00/04642	International filing date *(day/month/year)* 24 FEB 00
Applicant CASE WESTERN RESERVE UNIVERSITY	

MB
DOEE
8/18/00

1. ☐ REPLY DUE within _____ months/days from the above date of mailing

☐ NO REPLY DUE, however, see below _____

☒ IMPORTANT COMMUNICATION

☐ INFORMATION ONLY

2. COMMUNICATION:

The power of attorney is not signed by a person having apparent authority to sign on behalf of the organization indicated as applicant. This deficiency may be corrected by filing a power of attorney signed by a person having apparent authority to sign on behalf of the organization or by filing proof that the person signing the power of attorney has the authority to sign on behalf of the organization. Such proof may be in the form of a copy of a resolution of the board of directors, a provision of the bylaws, or a copy of a paper properly delegating authority to that person to sign the application on behalf of the organization.

Name and mailing address of the receiving Office Assistant Commissioner for Patent Box PCT Washington, D.C. 20231 Attn:RO/US Facsimile No. 703-305-3230	Authorized officer Paul F. Urrutia Telephone No. 703-305-3681

Form PCT/RO/132 (July 1992)

SAK

PATENT COOPERATION TREATY

RECEIVED

JUL 07 2000

From the RECEIVING OFFICE

To:
SARAH A. KAGAN
BANNER & WITCOFF, LTD
1001 G STREET, N.W.
ELEVENTH FLOOR
WASHINGTON, DC 20001 4597

PCT

BANNER & WITCOFF LTD.

COMMUNICATION REGARDING EXTENSION OF TIME LIMIT

(PCT Rule 26.2)

Date of mailing *(day/month/year)*	30 JUN 2000
Applicant's or agent's file reference: 3037.86704	IMPORTANT COMMUNICATION
International application No.: PCT/US00/04642	International filing date *(day/month/year)*: 24 FEB 00
Applicant: CASE WESTERN RESERVE UNIVERSITY	

1. In response to the applicant's request of 30 MAY 2000, the time limit for replying to:

   [X] the Invitation to Correct Defects (PCT/RO/106)

   [ ] (other) _____

   has been extended as follows:

   [X] extension of 1 (months) days from 28 APRIL 2000

   [X] extension until 28 MAY 2000

2. [ ] No extension of the time limit is granted and the time limit remains as previously set.

Name and mailing address of the receiving Office Assistant Commissioner for Patent Box PCT Washington, D.C. 20231 Attn:RO/US Facsimile No. 703-305-3230	Authorized officer Paul F. Urrutia Telephone No. 703-305-3681

Form PCT/RO/138 (July 1992)

03037.86704 CAK
Previously Cketed
Filed: 26 SEP 2000

PCT/US00/04642

PATENT COOPERATION TREATY

PCT

REVIEWED CH + Sequence Listing due 5-27-00

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

From the INTERNATIONAL BUREAU

To:

KAGAN, Sarah, A.
Banner & Witcoff, Ltd.
11th floor
1001 G Street, N.W.
Washington, DC 20001-4597
ETATS-UNIS D'AMERIQUE

MB 5/26/00

Date of mailing (day/month/year) 03 May 2000 (03.05.00)	
Applicant's or agent's file reference 3037.86704	**IMPORTANT NOTIFICATION**
International application No. PCT/US00/04642	International filing date (day/month/year) 24 February 2000 (24.02.00)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 24 February 1999 (24.02.99)
Applicant CASE WESTERN RESERVE UNIVERSITY et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
24 Febr 1999 (24.02.99)	60/121,495	US	11 Apri 2000 (11.04.00)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Somsak Thiphrakesone

Telephone No. (41-22) 338.83.38

003258973

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT RECEIVED

NOTIFICATION OF RECEIPT OF SEARCH COPY APR 2 0 2000

(PCT Rule 23) BANNER & WITCOFF LTD.

To:
BANNER & WITCOFF, LTD.
Attn. KAGAN, Sarah A.
1001 G Street, N.W.
Eleventh Floor
Washington, DC 20001-4597
UNITED STATES OF AMERICA

Date of mailing *(day/month/year)*	14/04/2000
Applicant's or agent's file reference: 3037.86704	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 00/04642	24/02/2000	24/02/1999

Applicant

CASE WESTERN RESERVE UNIVERSITY et al.

1. **Where the International Searching Authority and the Receiving Office are not the same office:**

The applicant is hereby notified that the search copy of the international application was received by this International Searching Authority on the date indicated below.

Where the International Searching Authority and the Receiving Office are the same office:

The applicant is hereby notified that the search copy of the international application was received on the date indicated below.

27/03/2000 *(date of receipt).*

2. ☐ The search copy was accompanied by a nucleotide and/or amino acid sequence listing in computer readable form.

3. **Time limit for establishment of International Search Report**

The applicant is informed that the time limit for establishing the International Search Report is 3 months from the date of receipt indicated above or 9 months from the priority date, whichever time limit expires later

4. A copy of this notification has been sent to the International Bureau and, where the first sentence of paragraph 1 applies, to the Receiving Office.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl Fax: (+31-70) 340-3016	ISA/EP

Form PCT/ISA/202 (July 1998)

PATENT COOPERATION TREATY

RECEIVED

From the RECEIVING OFFICE

To:
SARAH A. KAGAN
BANNER & WITCOFF, LTD
1001 G STREET, N.W.
ELEVENTH FLOOR
WASHINGTON, DC 20001 4597

PCT

MAR 2 8 2000

BANNER & WITCOFF LTD.

NOTIFICATION CONCERNING PAYMENT OF PRESCRIBED FEES

(PCT Rules 14, 15 and 16 and Administrative Instructions, Sections 304(a) and (b) and 323(b))

Date of mailing *(day/month/year)*	28 MAR 00
Applicant's or agent's file reference: 3037.86704	**PAYMENT DUE** see item 3 for time limits

International application No.	International filing date/Date of receipt *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/04642	24 FEB 00	24 FEB 99

Applicant: CASE WESTERN RESERVE UNIVERSITY

1. The applicant is hereby notified that this receiving Office has received:

☒ **the payment of all the prescribed fees, and** ☐ **an overpayment, which will be refunded in due course.**

☐ **no or insufficient payment of the prescribed fees and the applicant is hereby invited to pay the balance due, as summarized under item 2, within the time limit(s) indicated under item 3.**

2. **Fees and payment calculation:**

0.00	–	0.00	=	0.00
Total fees payable		Amount paid		Balance

☐ The details of the calculation are given in the Annex.

3. **Time limit(s) for payment and amount(s) payable (Rules 14.1, 15.4 and 16.1(f)):**

☐ within ONE MONTH from the date of receipt of the international application (**for the transmittal fee** (if any), **the search fee, the basic fee and the designation fee**). The amount payable for each fee is the amount applicable on the date of receipt of the international application.

☐ within ONE YEAR from the priority date (**only for the designation fee and only if this time limit expires later than the above time limit**).
— If the designation fee is paid within one month from the date of receipt of the international application, the amount payable is the amount applicable on that date of receipt.
— If the designation fee is paid within one year from the priority date but later than one month from the date of receipt of the international application, the amount payable is the amount applicable on the date of payment. The receiving Office should be consulted for the applicable amount.

☐ within 16 MONTHS from the priority date (only for the fee for priority document). The applicant's attention is drawn to the fact that the request made by the applicant under Rule 17.1(b) will be considered not to have been made unless the fee is paid within that time limit.

4. Additional observations *(if necessary)*:

☐ The search copy will not be transmitted to the International Searching Authority until the search fee is paid (therefore the start of the international search will be delayed) (Rule 23.1(a) and (b)).

Name and mailing address of the receiving Office	Authorized officer
Assistant Commissioner for Patent Box PCT Washington, D.C. 20231 Attn:RO/US Facsimile No. 703-305-3230	Paul F. Urrutia Telephone No. 703-305-3681

Form PCT/RO/102 (January 1999; reprint January 2000)

03037.86704
DOCKETED ecb
APR 06 2000
Correct Defect Due:
28 Apr 2000

DOCKETED
SEP 12 2000

WO 00/50591
PCT/US00/04642

PATENT COOPERATION TREATY

+ Article 19 Amend due 9-24-00

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
KAGAN, Sarah, A.
Banner & Witcoff, Ltd.
11th floor
1001 G Street, N.W.
Washington, DC 20001-4597
ETATS-UNIS D'AMERIQUE

RECEIVED
SEP 12 2000
BANNER & WITCOFF LTD.

Date of mailing (day/month/year) 31 August 2000 (31.08.00)	
Applicant's or agent's file reference 3037.86704	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/US00/04642	24 February 2000 (24.02.00)	24 February 1999 (24.02.99)

Applicant
CASE WESTERN RESERVE UNIVERSITY et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,
   GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,
   NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   31 August 2000 (31.08.00) under No. WO 00/50591

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

Dat'l Phase
Ext. 8-24-01

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

KAGAN, Sarah, A.
Banner & Witcoff, Ltd.
11th floor
1001 G Street, N.W.
Washington, DC 20001-4597
ETATS-UNIS D'AMERIQUE

OCT 19 2000

INFORMATION CONCERNING ELECTED OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

Date of mailing (day/month/year) 12 October 2000 (12.10.00)	
Applicant's or agent's file reference 3037.86704	**IMPORTANT INFORMATION**
International application No. PCT/US00/04642	**International filing date** (day/month/year) 24 February 2000 (24.02.00)
Priority date (day/month/year) 24 February 1999 (24.02.99)	
Applicant CASE WESTERN RESERVE UNIVERSITY et al	

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   AP :GH,GM,KE,LS,MW,SD,SL,SZ,TZ,UG,ZW
   EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
   National :AU,BG,CA,CN,CZ,DE,IL,JP,KP,KR,MN,NO,NZ,PL,RO,RU,SE,SK,US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

   EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
   OA :BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG
   National :AE,AL,AM,AT,AZ,BA,BB,BR,BY,CH,CR,CU,DK,DM,EE,ES,FI,GB,GD,GE,GH, GM,HR,HU,ID,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MW,MX,PT,SD, SG,SI,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing , if prescribed, a translation of the international application (Article 39(1)(a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3)(b) and Rule 74.1).

   Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

   The entry into the European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer: Juan Cruz
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

Form PCT/IB/332 (September 1997)

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent, with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line below:
IPEA/ EP

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty. 09/914213

The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION		Applicant's or agent's file reference 3037.86704
International application No. PCT/US00/04642	International filing date *(day/month/year)* (24.02.00) 24 February 2000	(Earliest) Priority date (*day/month/year*) (24.02.99) 24 February 1999
Title of invention **ENHANCERS OF CFTR CHLORIDE CHANNEL FUNCTIONS**		

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*	Telephone No.:
CASE WESTERN RESERVE UNIVERSITY School of Medicine 10900 Euclid Avenue Cleveland, Ohio 4406-4971 United States of America	Facsimile No.:
	Teleprinter No.:

State *(that is, country)* of nationality: US	State *(that is, country)* of residence: US

Name and address: *(Family name followed by given name; for legal entity, full official designation. The address must include postal code and name of country.)*

ADAMS, Lynn M.
c/o Case Western Reserve University
School of Medicine
10900 Euclid Avenue
Cleveland, Ohio 4406-4971
United States of America

State *(that is, country)* of nationality: US	State *(that is, country)* of residence: US

Name and address: *(Family name followed by given name; for legal entity, full official designation. The address must include postal code and name of country.)*

DAVIS, Pamela B.
c/o Case Western Reserve University
School of Medicine
10900 Euclid Avenue
Cleveland, Ohio 4406-4971
United States of America

State *(that is, country)* of nationality: US	State *(that is, country)* of residence: US

International application No.
PCT/US00/04642

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet should not to be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

MA, Jianjie
c/o Case Western Reserve University
School of Medicine
10900 Euclid Avenue
Cleveland, Ohio 4406-4971
United States of America

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

☐ Further applicants are indicated on another continuation sheet.

See Notes to the demand form

International application No.
PCT/US00/04642

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is [X] agent [ ] common representative

and [X] has been appointed earlier and represents the applicant(s) also for international preliminary examination.

[ ] is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

[ ] is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

KAGAN, Sarah A.
BANNER & WITCOFF, LTD.
1001 G Street, N.W.
Eleventh Floor
Washington, D.C. 20001
United States of America

Telephone No.:
(202) 508-9100

Facsimile No.:
(202) 508-9299

Teleprinter No.:
N/A

[ ] **Address for Correspondence**: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

   [X] the international application as originally filed

   the description [X] as originally filed
   [ ] as amended under Article 34

   the claims [X] as originally filed
   [ ] as amended under Article 19 (together with any accompanying statement)
   [ ] as amended under Article 34

   the drawings [X] as originally filed
   [ ] as amended under Article 34

2. [ ] The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. [X] The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired).*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH
[X] which is the language in which the international application was filed.
[ ] which is the language of a translation furnished for the purposes of international search.
[ ] which is the language of publication of the international application.
[ ] which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby elects **all eligible States** (*that is, all States which have been designated and which are bound by Chapter II of the PCT*) excluding the following States which the applicant wishes **not to elect**: _____

International application No.
PCT/US00/04642

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

				For International Preliminary Examining Authority use only	
				received	not received
1.	translation of international application	:	sheets	[ ]	[ ]
2.	amendments under Article 34	:	sheets	[ ]	[ ]
3.	copy (or, where required, translation) of amendments under Article 19	:	sheets	[ ]	[ ]
4.	copy (or, where required, translation) of statement under Article 19	:	sheets	[ ]	[ ]
5.	letter	:	sheets	[ ]	[ ]
6.	other *(specify)*	:	sheets	[ ]	[ ]

The demand is also accompanied by the item(s) marked below:

1. [X] fee calculation sheet
2. [ ] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [ ] nucleotide and/or amino acid sequence listing in computer readable form
6. [ ] other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

Sarah A. Kagan
Agent for the Applicant(s)

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. [ ] The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply.
   [ ] The applicant has been informed accordingly.
4. [ ] The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. [ ] Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

See Notes to the demand form

CHAPTER II

PCT

FEE CALCULATION SHEET

Annex to the Demand for international preliminary examination

For International Preliminary Examining Authority use only

International application No. PCT/US00/04642

Applicant's or agent's file reference 3037.86704

Applicant CASE WESTERN UNIVERSITY

Date stamp of the IPEA

Calculation of prescribed fees

1. Preliminary examination fee ..... EUR 1,533 [P]

2. Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)* ..... EUR 147 [H]

3. Total of prescribed fees
Add the amounts entered at P and H and enter total in the TOTAL box ..... EUR 1,680 TOTAL

Mode of Payment

[ ] authorization to charge deposit account with the IPEA (see below)

[ ] cheque

[ ] postal money order

[X] bank draft

[ ] cash

[ ] revenue stamps

[ ] coupons

[ ] other *(specify)*:

Deposit Account Authorization ***(this mode of payment may not be available at all IPEAs)***

The IPEA/ EP [ ] is hereby authorized to charge the total fees indicated above to my deposit account.

[ ] *(this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

N/A
Deposit Account Number

15 September 2000
Date *(day/month/year)*

Signature: Sarah A. Hagan, Reg. No.: 32,141

See Notes to the fee calculation sheet

Nat'l / SAK

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:
KAGAN, Sarah A.
BANNER & WITCOFF, LTD.
1001 G Street, N.W.
Eleventh Floor
Washington, D.C. 20001-4597
ETATS-UNIS D'AMERIQUE

RECEIVED
OCT 10 2000
BANNER & WITCOFF LTD

Nat'l Phase
Ext. 8-24-01

NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*	04. 10. 00
Applicant's or agent's file reference: 3037.86704	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 00/04642	24/02/2000	24/02/1999

Applicant: CASE WESTERN RESERVE UNIVERSITY et al.

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

   18/09/2000

2. This date of receipt is:

   ☒ the actual date of receipt of the demand by this Authority (Rule 61.1(b)).

   ☐ the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).

   ☐ the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. ☐ **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

   ☐ *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on: _____

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	MORENO R A Tel. (+49-89) 2399-2658

EUROPÄISCHES PATENTAMT · EUROPEAN PATENT OFFICE · OFFICE EUROPÉEN DES BREVETS

PATENT COOPERATION TREATY

SAK

by fax and post

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

KAGAN, Sarah A.
BANNER & WITCOFF, LTD.
1001 G Street, N.W.
Eleventh Floor
Washington, D.C. 20001-4597
ETATS-UNIS D'AMERIQUE

FAX: 202 508 9299

03037.86704

AM FEB 25 2001

BANNER & WITCOFF LTD.

Written Opinion
5-20-2001

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	20.02.2001
Applicant's or agent's file reference: 3037.86704	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/04642	24/02/2000	24/02/1999

International Patent Classification (IPC) or both national classification and IPC

C12N15/12

Applicant

CASE WESTERN RESERVE UNIVERSITY et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 24/06/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Kania, T

Formalities officer (incl. extension of time limits)
Büchler, S
Telephone No. +49 89 2399 8090

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-20 as originally filed

Claims, No.:

1-34 as originally filed

Drawings, sheets:

1/4-4/4 as received on 16/08/2000 with letter of 26/06/2000

Sequence listing part of the description, pages:

1-3, filed with the letter of 19-05-00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 17,19,20,30,32,33,

because:

   ☒ the said international application, or the said claims Nos. 17,19,20,30,32,33 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A written opinion cannot be drawn due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrialapplicability; citations and explanations supporting such statement

1. Statement
   Novelty (N) Claims 1-4,7:no

Inventive step (IS)	Claims	1-7:no
Industrial applicability (IA)	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

The document numbering (D1-D8) corresponds to their order of citation in the search report. Documents D4-D6 were not considered for this report.

Section III (non-establishment of opinion, Art.33(4), Rule 67.1 (IV) PCT).
For the assessment of the present claims 17, 19, 20, 30, 32, 33 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims.

Section V (novelty and inventive step, Art.33 PCT).
1. The present application investigates the role of the 22 amino acid negatively charged region ("NEG2", amino acids 817-38) of the cystic fibrosis transmembrane conductance regulator (CFTR) for chloride channel function.
It has been shown that deletion mutants in the NEG2 region are independent of phosphorylation by PKA. Mutant chloride channels show a lower but constitutive opening probability (Po).
Peptides have been created that span all or part of the NEG2 region according to SEQ ID NOs:1,2. In vitro, addition of greater than 0.44 uM of these peptides stimulates both w.t. and NEG2-deleted CFTR proteins, and increases the initial opening probability of the chloride channel by up to 200%.

2. Peptides of 18 or 22 amino acids having the sequence of SEQ ID:1 or SEQ ID:2 would be novel. Independent claim 1, however, is directed to an isolated polypeptide **comprising** these sequences. Since the NEG2 sequences of SEQ ID:1 and 2 are wild-type, said claim embraces the wild-type full-length CFTR protein as well as any fragment thereof embracing the NEG2 region (for fragments see e.g. D2 fragment RD2TM, figure 1; D3 fragment CFTR-deltaR/S660A).

Thus, claim 1 is not novel.

3. Dependent claims 2-4, 7 similarly lack novelty.

See eg. D8 which discloses a larger fragment of CFTR comprising part of the native transmembrane region (page 13 lines 6-17). In D2 and D3 non-phosphorylated fragments of CFTR have been disclosed (see D2 page 64 chapter 2.2.; D3 see abstract).

4. The fusion peptides as claimed in claims 5 and 6 appear to be novel over the prior art. However, it appears to be non-inventive in the light of prior art document D8 to replace one membrane-penetrating peptide by another without providing an additional technical effect.

5. The specific peptides having SEQ ID:1 (aa 817-34 of CFTR) or SEQ ID:2 (aa 817-38 of CFTR) furthermore appear to lack an inventive step with respect to D3.
D3 reports that an isolated CFTR fragment consisting of amino acids 645-834 of CFTR can stimulate CFTR activity of wild-type and mutant CFTR channels.
In cited document D1 the NEG2 region and sequence (aa 817-38) is disclosed. Furthermore, the document describes NEG2 deletion mutants, and their effects on channel function. The probable importance of NEG2 for chloride channel opening is addressed.
As the domain identified by D3 is still relatively large, it would have been obvious for the skilled person having knowledge of D3 to further delineate the active region according to the NEG2 region of D1, and thus arrive at peptides having SEQ ID NOs:1 or 2.

The subject-matter of claims 1-7 would thus lack an inventive step, *even if these claims were to be limited to the specific short peptides of SEQ ID NOs:1, 2.*

6. Independent claims 8 and 21 disclose methods for the activation of CFTR proteins employing polypeptides **comprising** the peptides defined as SEQ ID NO:1 and 2. Said methods have not been described in the prior art, and the claims consequently have to be considered as novel.

In the prior art the use of larger fragments of CFTR comprising the NEG2 region has been disclosed (see e.g. D2, D3, D8). Those peptides have a different effect on wild-type channels than R-deletion mutants, and their function is dependent on the phosphorylation state. The present peptides, however, appear to have a stimulating effect on both wild-type and mutant CFTR, and are independent of phosphorylation. Furthermore, the mere knowledge of functionally interesting domains in the CFTR molecule and the obvious action of creating peptides spanning such domains and using them in methods already described in the prior art (see D2, D3, D8), does not suggest the activating effect created by such peptides.

Thus, the claimed methods restricted to the actual peptides of SEQ ID NOs:1, 2, could be considered inventive over the available prior art.

7. A lack of sufficiency of disclosure is, however, seen for independent claims 8 and 21 in their present unduly broad formulation, see section VII hereinbelow.

Section VII

1. Due to the inappropriate use of the term **"comprising"**, the subject-matter of independent claims 8 and 21 is not sufficiently disclosed over the entire width of the claims, since the technical effect claimed would not be obtained with e.g. the complete CFTR protein or larger fragments thereof (see e.g. D2 pages 65/66 part 3.2).

2. The description of the drawings on page 4 does not refer to the correct figures in the case of figure 1 and figure 2.

Section VIII (Clarity, Art. 6 PCT)

1. Claims 1, 8, 21 are verbally unclear. These claims refer to "a polypeptide comprising a portion ... of between 10 and 100 amino acids, said portion comprising 18 amino acids...". The examiner does not understand how a fragment of 10 amino acids can "comprise" 18 amino acids.

2. Claim 16 refers to a homepage. Claims should be clear in themself and not rely on references to the description or other documents to define the claimed matter (Rule 6.2(a) PCT). As the contents of a homepage are not necessarily stable over time a reference to a homepage is particularly unsuitable to define the claimed matter. Moreover, the link per se appears to be invalid.

ATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
3037.86704		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/04642	24/02/2000	24/02/1999
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant CASE WESTERN RESERVE UNIVERSITY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/09/2000	19.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Kania, T Telephone No. +49 89 2399 7703

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/04642

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-20 as originally filed

Claims, No.:

1-34 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

Sequence listing part of the description, pages:

1-3, filed with the letter of 19-05-00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 17,19,20,30,32,33.

because:

   ☒ the said international application, or the said claims Nos. 17,19,20,30,32,33 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5,6,8-16,18,21-29,31,34
	No:	Claims	1-4,7
Inventive step (IS)	Yes:	Claims	8-16,18,21-29,31,34
	No:	Claims	1-7
Industrial applicability (IA)	Yes:	Claims	1-16,18,21-29,31,34
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

The document numbering (D1-D8) corresponds to their order of citation in the search report. Documents D4-D6 were not considered for this report.

Section III (non-establishment of opinion, Art.33(4), Rule 67.1 (IV) PCT).
For the assessment of the present claims 17, 19, 20, 30, 32, 33 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims.

Section V (novelty and inventive step, Art.33 PCT).
1. The present application investigates the role of the 22 amino acid negatively charged region ("NEG2", amino acids 817-38) of the cystic fibrosis transmembrane conductance regulator (CFTR) for chloride channel function.
It has been shown that deletion mutants in the NEG2 region are independent of phosphorylation by PKA. Mutant chloride channels show a lower but constitutive opening probability (Po).
Peptides have been created that span all or part of the NEG2 region according to SEQ ID NOs:1,2. In vitro, addition of greater than 0.44 uM of these peptides stimulates both w.t. and NEG2-deleted CFTR proteins, and increases the initial opening probability of the chloride channel by up to 200%.

2. Peptides of 18 or 22 amino acids having the sequence of SEQ ID:1 or SEQ ID:2 would be novel. Independent claim 1, however, is directed to an isolated polypeptide comprising these sequences. Since the NEG2 sequences of SEQ ID:1 and 2 are wild-type, said claim embraces the wild-type full-length CFTR protein as well as any fragment thereof embracing the NEG2 region (for fragments see e.g. D2 fragment RD2TM, figure 1; D3 fragment CFTR-deltaR/S660A).

Thus, claim 1 is not novel.

3. Dependent claims 2-4, 7 similarly lack novelty.

See eg. D8 which discloses a larger fragment of CFTR comprising part of the native transmembrane region (page 13 lines 6-17). In D2 and D3 non-phosphorylated fragments of CFTR have been disclosed (see D2 page 64 chapter 2.2.; D3 see abstract).

4. The fusion peptides as claimed in claims 5 and 6 appear to be novel over the prior art. However, it appears to be non-inventive in the light of prior art document D8 to replace one membrane-penetrating peptide by another without providing an additional technical effect.

5. The specific peptides having SEQ ID:1 (aa 817-34 of CFTR) or SEQ ID:2 (aa 817-38 of CFTR) furthermore appear to lack an inventive step with respect to D3.
D3 reports that an isolated CFTR fragment consisting of amino acids 645-834 of CFTR can stimulate CFTR activity of wild-type and mutant CFTR channels.
In cited document D1 the NEG2 region and sequence (aa 817-38) is disclosed. Furthermore, the document describes NEG2 deletion mutants, and their effects on channel function. The probable importance of NEG2 for chloride channel opening is addressed.
As the domain identified by D3 is still relatively large, it would have been obvious for the skilled person having knowledge of D3 to further delineate the active region according to the NEG2 region of D1, and thus arrive at peptides having SEQ ID NOs:1 or 2.

The subject-matter of claims 1-7 would thus lack an inventive step, even if these claims were to be limited to the specific short peptides of SEQ ID NOs:1, 2.

6. Independent claims 8 and 21 disclose methods for the activation of CFTR proteins employing polypeptides comprising the peptides defined as SEQ ID NO:1 and 2.
Said methods have not been described in the prior art, and the claims consequently have to be considered as novel.

In the prior art the use of larger fragments of CFTR comprising the NEG2 region has been disclosed (see e.g. D2, D3, D8). Those peptides have a different effect on wild-type channels than R-deletion mutants, and their function is dependent on the phosphorylation state. The present peptides, however, appear to have a stimulating effect on both wild-type and mutant CFTR, and are independent of phosphorylation. Furthermore, the mere knowledge of functionally interesting domains in the CFTR molecule and the obvious action of creating peptides spanning such domains and using them in methods already described in the prior art (see D2, D3, D8), does not suggest the activating effect created by such peptides.

Thus, the claimed methods restricted to the actual peptides of SEQ ID NOs:1, 2, could be considered inventive over the available prior art.

7. A lack of sufficiency of disclosure is, however, seen for independent claims 8 and 21 in their present unduly broad formulation, see section VII hereinbelow.

Section VII

1. Due to the inappropriate use of the term "comprising", the subject-matter of independent claims 8 and 21 is not sufficiently disclosed over the entire width of the claims, since the technical effect claimed would not be obtained with e.g. the complete CFTR protein or larger fragments thereof (see e.g. D2 pages 65/66 part 3.2).

2. The description of the drawings on page 4 does not refer to the correct figures in the case of figure 1 and figure 2.

Section VIII (Clarity, Art. 6 PCT)

1. Claims 1, 8, 21 are verbally unclear. These claims refer to "a polypeptide comprising a portion ... of between 10 and 100 amino acids, said portion comprising 18 amino acids...". The examiner does not understand how a fragment of 10 amino acids can "comprise" 18 amino acids.

2. Claim 16 refers to a homepage. Claims should be clear in themself and not rely on references to the description or other documents to define the claimed matter (Rule 6.2(a) PCT). As the contents of a homepage are not necessarily stable over time a reference to a homepage is particularly unsuitable to define the claimed matter. Moreover, the link per se appears to be invalid.

PATENT COOPERATION TREATY

SAK

fax and post

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

03037.86704
RECEIVED
JUN 26 2001
BANNER & WITCOFF, LTD.

KAGAN, Sarah A.
BANNER & WITCOFF, LTD.
1001 G Street, N.W.
Eleventh Floor
Washington, D.C. 20001-4597
ETATS-UNIS D'AMERIQUE
FAX NO: 202 508 9299

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	19.06.2001
Applicant's or agent's file reference 3037.86704	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/04642	24/02/2000	24/02/1999

Applicant
CASE WESTERN RESERVE UNIVERSITY et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Büchler, S Tel.+49 89 2399-8090

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
3037.86704		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/04642	24/02/2000	24/02/1999
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant CASE WESTERN RESERVE UNIVERSITY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/09/2000	19.06.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Kania, T Telephone No. +49 89 2399 7703

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/04642

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-20 as originally filed

Claims, No.:

1-34 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

Sequence listing part of the description, pages:

1-3, filed with the letter of 19-05-00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 17,19,20,30,32,33.

because:

   ☒ the said international application, or the said claims Nos. 17,19,20,30,32,33 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5,6,8-16,18,21-29,31,34
	No:	Claims	1-4,7
Inventive step (IS)	Yes:	Claims	8-16,18,21-29,31,34
	No:	Claims	1-7
Industrial applicability (IA)	Yes:	Claims	1-16,18,21-29,31,34
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

The document numbering (D1-D8) corresponds to their order of citation in the search report. Documents D4-D6 were not considered for this report.

Section III (non-establishment of opinion, Art.33(4), Rule 67.1 (IV) PCT).
For the assessment of the present claims 17, 19, 20, 30, 32, 33 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims.

Section V (novelty and inventive step, Art.33 PCT).
1. The present application investigates the role of the 22 amino acid negatively charged region ("NEG2", amino acids 817-38) of the cystic fibrosis transmembrane conductance regulator (CFTR) for chloride channel function.
It has been shown that deletion mutants in the NEG2 region are independent of phosphorylation by PKA. Mutant chloride channels show a lower but constitutive opening probability (Po).
Peptides have been created that span all or part of the NEG2 region according to SEQ ID NOs:1,2. In vitro, addition of greater than 0.44 uM of these peptides stimulates both w.t. and NEG2-deleted CFTR proteins, and increases the initial opening probability of the chloride channel by up to 200%.

2. Peptides of 18 or 22 amino acids having the sequence of SEQ ID:1 or SEQ ID:2 would be novel. Independent claim 1, however, is directed to an isolated polypeptide comprising these sequences. Since the NEG2 sequences of SEQ ID:1 and 2 are wild-type, said claim embraces the wild-type full-length CFTR protein as well as any fragment thereof embracing the NEG2 region (for fragments see e.g. D2 fragment RD2TM, figure 1; D3 fragment CFTR-deltaR/S660A).

Thus, claim 1 is not novel.

3. Dependent claims 2-4, 7 similarly lack novelty.

See eg. D8 which discloses a larger fragment of CFTR comprising part of the native transmembrane region (page 13 lines 6-17). In D2 and D3 non-phosphorylated fragments of CFTR have been disclosed (see D2 page 64 chapter 2.2.; D3 see abstract).

4. The fusion peptides as claimed in claims 5 and 6 appear to be novel over the prior art. However, it appears to be non-inventive in the light of prior art document D8 to replace one membrane-penetrating peptide by another without providing an additional technical effect.

5. The specific peptides having SEQ ID:1 (aa 817-34 of CFTR) or SEQ ID:2 (aa 817-38 of CFTR) furthermore appear to lack an inventive step with respect to D3.
D3 reports that an isolated CFTR fragment consisting of amino acids 645-834 of CFTR can stimulate CFTR activity of wild-type and mutant CFTR channels.
In cited document D1 the NEG2 region and sequence (aa 817-38) is disclosed. Furthermore, the document describes NEG2 deletion mutants, and their effects on channel function. The probable importance of NEG2 for chloride channel opening is addressed.
As the domain identified by D3 is still relatively large, it would have been obvious for the skilled person having knowledge of D3 to further delineate the active region according to the NEG2 region of D1, and thus arrive at peptides having SEQ ID NOs:1 or 2.

The subject-matter of claims 1-7 would thus lack an inventive step, even if these claims were to be limited to the specific short peptides of SEQ ID NOs:1, 2.

6. Independent claims 8 and 21 disclose methods for the activation of CFTR proteins employing polypeptides comprising the peptides defined as SEQ ID NO:1 and 2.
Said methods have not been described in the prior art, and the claims consequently have to be considered as novel.

In the prior art the use of larger fragments of CFTR comprising the NEG2 region has been disclosed (see e.g. D2, D3, D8). Those peptides have a different effect on wild-type channels than R-deletion mutants, and their function is dependent on the phosphorylation state. The present peptides, however, appear to have a stimulating effect on both wild-type and mutant CFTR, and are independent of phosphorylation. Furthermore, the mere knowledge of functionally interesting domains in the CFTR molecule and the obvious action of creating peptides spanning such domains and using them in methods already described in the prior art (see D2, D3, D8), does not suggest the activating effect created by such peptides.

Thus, the claimed methods restricted to the actual peptides of SEQ ID NOs:1, 2, could be considered inventive over the available prior art.

7. A lack of sufficiency of disclosure is, however, seen for independent claims 8 and 21 in their present unduly broad formulation, see section VII hereinbelow.

Section VII

1. Due to the inappropriate use of the term "comprising", the subject-matter of independent claims 8 and 21 is not sufficiently disclosed over the entire width of the claims, since the technical effect claimed would not be obtained with e.g. the complete CFTR protein or larger fragments thereof (see e.g. D2 pages 65/66 part 3.2).

2. The description of the drawings on page 4 does not refer to the correct figures in the case of figure 1 and figure 2.

Section VIII (Clarity, Art. 6 PCT)

1. Claims 1, 8, 21 are verbally unclear. These claims refer to "a polypeptide comprising a portion ... of between 10 and 100 amino acids, said portion comprising 18 amino acids...". The examiner does not understand how a fragment of 10 amino acids can "comprise" 18 amino acids.

2. Claim 16 refers to a homepage. Claims should be clear in themself and not rely on references to the description or other documents to define the claimed matter (Rule 6.2(a) PCT). As the contents of a homepage are not necessarily stable over time a reference to a homepage is particularly unsuitable to define the claimed matter. Moreover, the link per se appears to be invalid.

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PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

International Application No.
International Filing Date
Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* 3037.86704

Box No. I TITLE OF INVENTION

ENHANCERS OF CFTR CHLORIDE CHANNEL FUNCTION

09/914213

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

Case Western Reserve University
School of Medicine
10900 Euclid Avenue
Cleveland, Ohio 4406-4971

[ ] This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No. N/A

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: [ ] all designated States [ ] the United States of America only [X] all designated States except the United States of America [ ] the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ADAMS, Lynn M.
c/o Case Western Reserve University
School of Medicine
10900 Euclid Avenue
Cleveland, Ohio 4406-4971

This person is:

[ ] applicant only

[X] applicant and inventor

[ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: [ ] all designated States [X] the United States of America only [ ] all designated States except the United States of America [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: [X] agent [ ] common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

KAGAN, Sarah A.
BANNER & WITCOFF, LTD.
1001 G Street, N.W.
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US

Telephone No. (202) 508-9100

Facsimile No. (202) 508-9299

Teleprinter No. N/A

[ ] **Address for correspondence**: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTORS

If non of th following sub-boxes is used, this sh et should not be included in th request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

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This person is:
[ ] applicant only
[X] applicant and inventor
[ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: [ ] all designated States [X] the United States of America only [ ] all designated States except the United States of America [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

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This person is:
[ ] applicant only
[X] applicant and inventor
[ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: [ ] all designated States [X] the United States of America only [ ] all designated States except the United States of America [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
[ ] applicant only
[ ] applicant and inventor
[ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

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This person is applicant for the purposes of: [ ] all designated States [ ] the United States of America only [ ] all designated States except the United States of America [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
[ ] applicant only
[ ] applicant and inventor
[ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: [ ] all designated States [ ] the United States of America only [ ] all designated States except the United States of America [ ] the States indicated in the Supplemental Box

[ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a)*(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

[X] **AP** **ARIPO Patent:** **GH** Ghana, **GM** Gambia, **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SL** Sierra Leone, **SZ** Swaziland, **TZ** United Republic of Tanzania, **UG** Uganda, **ZW** Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

[X] **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

[X] **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **CY** Cyprus, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

[X] **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **GW** Guinea-Bissau, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

[X] **AE** United Arab Emirates
[X] **AL** Albania .....
[X] **AM** Armenia .....
[X] **AT** Austria .....
[X] **AU** Australia .....
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[X] **BA** Bosnia and Herzegovina .....
[X] **BB** Barbados
[X] **BG** Bulgaria .....
[X] **BR** Brazil .....
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[X] **SL** Sierra Leone .....
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[ ] .....

Supplemental Box *If the Supplemental Box is not used, this sheet should not be included in the request.*

*1. If, in any of the Boxes, **the space is insufficient** to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient,in particular:*

*(i) **if more than two persons are involved as applicants and/or inventors** and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;*

*(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication **"the States indicated in the Supplemental Box"** is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*

*(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, **the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America**: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*

*(iv) if, in addition to the agent(s) indicated in Box No. IV, there are **further agents**: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*

*(v) if, in Box No. V, the name of an State (or OAPI) is accompanied by the indication **"patent of addition,"** or **"certificate of addition,"** or if, in Box No. V, the name of the United States of America is accompanied by an indication **"continuation"** or **"continuation-in-part"**: in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*

*(vi) if, in Box No. VI, there are **more than three earlier applications whose priority is claimed**: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;*

*(vii) if, in Box No. VI, **the earlier application is an ARIPO application**: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.*

*2. If, with regard to the **precautionary designation statement** contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.*

*3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning **non-prejudicial disclosures or exceptions to lack of novelty**: in such case write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.*

Continuation of Box No. IV:

ALTHERR, Robert F.
BANNER, Donald W.
BANNER, Pamela I.
BECKET, William W.
FEDEROCHKO, Gary D.
FISHER, William J.
HONG, Patricia E.
HOSCHEIT, Dale H.
JACKSON, Thomas H.
KAGAN, Sarah A.
McKIE, Edward F. Jr.
MEDLOCK, Nina L.
NIEGOWSKI, James A.
PETERSON, Thomas L.
POTENZA, Joseph M.
SKERPON, Joseph M.
SCHAD, Steven P.
WOLFFE, Franklin D.
WOLFFE, Susan A.
WRIGHT, Bradley C.

All members of the firm of BANNER & WITCOFF, LTD. at the address, telephone and telefacsimile numbers as indicated in Box No. IV.

Continuation of Box No. V:

US 60/121.495 filed 24 February 1999 (24.02.99)

Box No. VI PRIORITY CLAIM [ ] Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 24 February 1999 24.02.99	60/121,495	US		
item (2)				
item (3)				

[X] The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is th receiving Office)* identified above as item(s): 1

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection olf Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used)*:
ISA/EP

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*
Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets**:

request	:	5 sheets
description (excluding sequence listing part)	:	20 sheets
claims	:	4 sheets
abstract	:	1 sheet
drawings	:	4 sheets
sequence listing part of description	:	1 sheet
Total number of sheets	:	35 sheets

This international application is **accompanied by** the item(s) marked below:

1. **[X] fee calculation sheet (duplicate)**
2. [ ] separate signed power of attorney
3. [ ] copy of general power of attorney; reference number, if any:
4. [ ] statement explaining lack of signature
5. [ ] priority document(s) identified in Box No. VI as item(s):
6. [ ] translation of international application into (language):
7. [ ] separate indications concerning deposited microorganism or other biological material
8. [ ] nucleotide and/or amino acid sequence listing in computer readable form
9. **[X] other *(specify)*: Transmittal**

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Lisa M. Hennessey

for Sarah A. Kagan
Agent for the Applicant(s)

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings:
   [ ] received:
   [ ] not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA/EP
6. [ ] Transmittal of search copy delayed until search fee is paid

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Date of receipt of the record copy by the International Bureau:

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FEE CALCULATION SHEET
Annex to the Request

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference 3037.86704

Applicant
CASE WESTERN RESERVE UNIVERSITY

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE	240 | T	
2. SEARCH FEE	990 | S	

International search to be carried out by **EP**
(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee
The international application contains **35** sheets.

first 30 sheets	b_1 | 427	
5 remaining sheets X 10.00 additional amount =	b_2 | 50	
Add amounts entered at b_1 and b_2 and enter total at B	477 | B	

Designation Fees
The international application contains **ALL** designations.

8 number of designation fees x 92.00 amount of designation fee payable (*maximum 8*) =	736 | D	
Add amounts entered at B and D and enter total at I	1213 | I	

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)

4. FEE FOR PRIORITY DOCUMENT *(if applicable)*	15 | P	
5. TOTAL FEES PAYABLE Add amounts entered at T, S, I and P, and enter total in the TOTAL box	**TOTAL: USD $** 2458	

[ ] The designation fee is not paid at this time.

MODE OF PAYMENT

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19-0733 Deposit Account Number	24 February 2000 Date (*day/month/year*)	Lydia M. Hennessey, Reg. No. 42,653 for Signature: Sarah A. Kagan, Reg. No.: 32,141

Form PCT/RO/101 (Annex) (January 2000)

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PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/12, 15/62, C07K 14/47, A61K 38/17, 48/00

A1

(11) International Publication Number: WO 00/50591

(43) International Publication Date: 31 August 2000 (31.08.00)

(21) International Application Number: PCT/US00/04642

(22) International Filing Date: 24 February 2000 (24.02.00)

(30) Priority Data:
60/121,495 24 February 1999 (24.02.99) US

(71) Applicant *(for all designated States except US)*: CASE WESTERN RESERVE UNIVERSITY [US/US]; School of Medicine, 10900 Euclid Avenue, Cleveland, OH 4406–4946 (US).

(72) Inventors; and

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(54) Title: ENHANCERS OF CFTR CHLORIDE CHANNEL FUNCTION

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B. FIELDS SEARCHED

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ADAMS L M ET AL: "Deletion of a negatively charged region (a.a 817-838) from the R domain of CFTR alters PKA-dependent regulation of the CFTR channel." BIOPHYSICAL JOURNAL, vol. 74, no. 2 PART 2, February 1998 (1998-02), page A344 XP000923128 Forty-second Annual Meeting of the Biophysical Society;Kansas City, Missouri, USA; February 22-26, 1998 ISSN: 0006-3495 abstract --- -/--	1,2,7

[X] Further documents are listed in the continuation of box C.

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INTERNATIONAL SEARCH REPORT

information on patent family members

Inte[redacted]al Application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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ENHANCERS OF CFTR CHLORIDE CHANNEL FUNCTION

This invention was made with government support under RO1 HL/DK 49003, P30 DK27651 and RO1 DK51770 awarded by the National Institute of Health. The government has certain rights in the invention.

TECHNICAL FIELD OF THE INVENTION

This invention is related to the field of cystic fibrosis. More particularly, it is related to the area of therapeutic treatments and drug discovery for treating cystic fibrosis.

BACKGROUND OF THE INVENTION

Defects in CFTR, a chloride channel located in the apical membrane of epithelial cells, are associated with the common genetic disease, cystic fibrosis (Quinton, 1986, Welsh and Smith, 1993, Zielenski and Tsui, 1995). CFTR is a 1480 amino acid protein that is a member of the ATP binding cassette (ABC) transporter family (Riordan et al., 1989, Higgins, 1992). Each half of CFTR contains a transmembrane domain and a nucleotide binding fold (NBF), and the two halves are connected by a regulatory, or R domain. The R domain is unique to CFTR and contains several consensus PKA phosphorylation sites (Cheng et al., 1991, Picciotto et al., 1992).

Opening of the CFTR channel is controlled by PKA phosphorylation of

serine residues in the R domain (Tabcharani et al., 1991, Bear et al., 1992) and ATP binding and hydrolysis at the NBFs (Anderson et al., 1991, Gunderson and Kopito, 1995). Phosphorylation adds negative charges to the R domain, and introduces global conformational changes reflected by the reduction in the α-helical content of the R domain protein (Dulhanty and Riordan, 1994). Thus, electrostatic and/or allosteric changes mediated by phosphorylation are likely to be responsible for interactions between the R domain and other CFTR domains that regulate channel function (Rich et al., 1993, Gadsby and Nairn, 1994).

Rich et al., 1991 showed that deletion of amino acids 708-835 from the R domain (ΔR-CFTR), which removes most of the PKA consensus sites, renders the CFTR channel PKA independent, but the open probability of ΔR-CFTR is one-third that of the wild type channel and does not increase upon PKA phosphorylation (Ma et al., 1997, Winter and Welsh, 1997). Thus, it is possible that deletion of the R domain removes both inhibitory and stimulatory effects conferred by the R domain on CFTR chloride channel function. This conclusion is supported by studies that show that addition of exogenous unphosphorylated R domain protein (amino acids 588-858) to wt-CFTR blocks the chloride channel (Ma et al., 1996), suggesting that the unphosphorylated R domain is inhibitory. Conversely, exogenous phosphorylated R domain protein (amino acids 588-855 or 645-834) stimulated the ΔR-CFTR channel, suggesting that the phosphorylated R domain is stimulatory (Ma et al., 1997, Winter and Welsh, 1997). Therefore, it appears that the manifest activity (stimulatory or inhibitory) depends on the phosphorylation state of the R domain.

About 25% of the known 700 mutations in CFTR produce a mutant CFTR

protein which is properly transported to the apical membrane of epithelial cells but have only low level, residual channel activity. There is a need in the art for agents which can boost the level of channel activity in those mutants having low level activity.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide an isolated polypeptide useful for enhancing the open probability of CFTR chloride channels.

It is another object of the present invention to provide a method of activating a CFTR protein to enhance its open probability.

These and other objects of the invention are achieved by providing one or more of the embodiments described below. In one embodiment of the invention an isolated polypeptide is provided. The polypeptide comprises a portion of CFTR (cystic fibrosis transmembrane conductance regulator) protein of between 10 and 100 amino acids, said portion comprising 18 amino acids as shown in SEQ ID NO: 1.

In another embodiment of the invention a method is provided for activating a CFTR protein. A polypeptide is applied to a CFTR protein which forms a cAMP regulated chloride channel. The polypeptide consists of a portion of CFTR protein which comprises 18 amino acids as shown in SEQ ID NO: 1, whereby the open probability of the channel formed by the CFTR increases by at least 25%.

According to another aspect of the invention a method is provided for activating a CFTR protein. A polypeptide is applied to a CFTR protein which forms a cAMP regulated chloride channel. The polypeptide consists of a portion of CFTR protein which comprises 22 amino acids as shown in SEQ ID NO: 2, whereby the

open probability of the channel formed by the CFTR increases by at least 25%.

The present invention thus provides the art with reagents and tools for enhancing function of channels which are defective in cystic fibrosis patients.

DETAILED DESCRIPTION OF THE DRAWINGS

Figure 1. Deletion of Negatively Charged Regions from the R Domain Results in Expression of Mature Glycosylated, Phosphorylatable CFTR Proteins

(Figure 1A) Sequences of NEG1 and NEG2 within the R domain. Residues where mutations have been identified in the CFTR cDNA are underlined (E822K, E826K, D836Y).

(Figure 1B) NEG2 is predicted to form an amphipathic α-helix as determined by secondary structure determination (Geourjon and Deleage, 1995, Rost and Sander, 1993, Rost and Sander, 1994) and illustrated in this space filling model. Negatively charged residues are colored pink, and the positively charged lysine is colored green.

(Figure 1C) In vitro phosphorylation of wt-(lane 1), ΔNEG1- (lane 2) and ΔNEG2-CFTR (lane 3) by PKA in the presence of γ-^{32}P-ATP. Both the core (band B) and fully glycosylated (band C) forms of all three CFTR molecules are phosphorylated.

Figure 2. ΔNEG2-CFTR Forms a Chloride Channel that is Unregulated by PKA

(Figure 2A) Single channel currents of wt, ΔNEG1- and ΔNEG2-CFTR incorporated into the lipid bilayer. While activities of wt-and ΔNEG1-CFTR absolutely require the presence of PKA in the *cis*-intracellular solution, the ΔNEG2-CFTR channel opens without PKA phosphorylation.

(Figure 2B) Diary plot of ΔNEG2-CFTR channel open probability versus time shows

that addition of 200 units/ml of PKA, a maximally stimulating concentration, does not affect channel activity. The dashed line indicates the average open probability for each segment of the experiment. Channels were recorded at –100 mV.

Figure 3. The Synthetic NEG2 Peptide both Stimulates and Inhibits CFTR

(Figure 3A) Diary plot (open probability versus time) of a wt-CFTR channel illustrating the effect of the NEG2 peptide on the open probability of the channel in the planar lipid bilayer. The concentration of synthetic NEG2 in the *cis*-intracellular solution is indicated above the plot.

(Figure 3B) Single channel currents from the wt-CFTR channel were acquired at –80 mV at the points indicated in A. The *cis*-intracellular solution contained 2 mM ATP and 50 units PKA/ml.

(Figure 3C) Single channel trace from ΔNEG2-CFTR incorporated into the lipid bilayer membrane. Traces were acquired at –80 mV. The *cis*-solution contained 2 mM ATP and no PKA. The top two traces were acquired before synthetic NEG2 peptide addition, with the second trace being an expansion of the first. In the bottom two traces, 0.44 μM of the NEG2 peptide has been added and stimulation is observed. The closed time visibly decreases after peptide addition.

Figure 4. NEG2 Enhances CFTR Channel Activity by Increasing the Opening Rate of the Channel

Histograms of open and closed events of the wt-CFTR channel at –80 mV were generated without peptide (control, left panel) and with 4.4 μM NEG2 peptide in the *cis*-solution (right panel).

(Figure 4A) The open time histograms contain a single exponential component with a time constant of 124 ms (control) and 105 ms (peptide-stimulated).

(Figure 4B) The closed time histograms contain a fast component and multiple slow components.

(Figure 4C) The closed-burst duration histograms were constructed using a delimiter of 40 ms (represented by the arrow in B). The solid lines in C represent the fit according to the double exponential equation $y = P_2*\exp[t-\alpha\text{-}\exp\alpha t-\alpha)]_2 + P_3*\exp[t-\alpha_3\text{-}\exp(t-\alpha_3)]$ where $\alpha_2 = \log \tau_{o2} \alpha_3 = \log \tau_{t3} P_2$ probability of the intermediate closed component, and P_3 = probability of the long closed component. The best fit parameters are $P_2 = 0.811$, $\tau_{c2} = 459$ ms, $P_3 = 0.189$, $\tau_{c3} = 2494$ ms (control); $P_2 = 0.957$, $\tau_{c2} = 105$ ms, $P_3 = 0.043$, $\tau_{c3} = 1652$ ms (peptide-stimulated).

DETAILED DESCRIPTION OF THE INVENTION

It is a discovery of the present inventors that negatively charged amino acids at the carboxyl terminal of the R domain (817-838, NEG2) is involved in both the stimulatory and inhibitory functions of the R domain on the chloride channel. Moreover, a polypeptide which contains this portion of the CFTR amino acid sequence can be used to enhance the open probability of both wild-type and minimally active mutant CFTR protein.

The isolated polypeptide according to the invention consists of a portion of CFTR (cystic fibrosis transmembrane conductance regulator) protein. The portion preferably contains at least 18 amino acids as shown in SEQ ID NO: 1. However, fewer amino acid residues of the sequence may be used if they retain the channel enhancing function described herein for the 18 and 22 residue polypeptides. See also SEQ ID NO: 2. Thus the polypeptide may be from about 10 or 15 amino acid residues up to about 30 or even 100 amino acid residues. An isolated polypeptide may be synthetic or made in a

recombinant organism. It may be a proteolytic cleavage product of a larger primary expression product, including full-length, wild-type CFTR. Preferably the polypeptide will be free of full-length CFTR. The polypeptide will preferably be free of other proteins and polypeptides as well. However, it may be desirable that the polypeptide be fused to another polypeptide to provide additional functional properties. For example, fusion to another protein such as keyhole limpet hemocyanin would be used to increase immunogenicity. Another desirable fusion partner is a membrane-penetrating peptide. Such peptides include VP-22 (SEQ ID NO: 3), as well as the peptides shown in SEQ ID NO: 4 and SEQ ID NO: 5. Such peptides can be used to facilitate the uptake by target cells of the polypeptide.

The polypeptides of the present invention can be used to enhance the function of wild-type or minimally active mutant CFTR proteins. The polypeptide functions to decrease the closed time of the channels formed by CFTR. A polypeptide can be applied to the CFTR protein in any context. It can be applied *in vitro* or *in vivo*. If *in vitro* it can be to CFTR in cultured cells or to planar bilayer membranes containing CFTR. If *in vivo,* the polypeptide can be applied directly to airway epithelial cells. Such application can be by any means known in the art, including but not limited to using a gargle or a nebulizer to deliver aerosolized polypeptide to the target cells. In addition, the peptide can be delivered in an indirect mode, by delivering a gene construct to the airway epithelial cells, which when taken up by the cells causes them to express the polypeptide. The delivery of the polypeptide to the CFTR preferably increases the open probability of the channel formed by the CFTR by at least 25%. More preferably it increases the open probability by at least 50%, at least 75%, at least 100%, at least 125%, at least 150%, or at least 200%.

A CFTR construct comprises a nucleic acid sequence encoding the amino acid sequence shown in SEQ ID NO: 1. A suitable promoter for expression in lung epithelia is also desirable. Many such promoters are known in the art, and any can be used as appropriate for a particular application.

It is believed that the administration of the polypeptide of the present invention will be the most useful in treatment of a class of mutants which produce CFTR proteins which are properly delivered to the plasma membrane but which are only residually or minimally active. Known mutants of CFTR are listed at http://www.genet.sickkids.on.ca/cftr-cgi-bin/fulltable. One can determine that a particular CFTR mutant is fully processed and reaches the plasma membrane in a Western blot assay using antibody against CFTR. Fully processed mutants achieve mature glycosylation status and appear on the gel as "band C and band B" whereas mutants that are retained in the endoplasmic reticulum are not fully glycosylated and show only "band B". See Example 2, below and Figure 1C.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLES

Example 1: Deletion of a negatively charged region (a.a. 817-838) from the R domain of CFTR alters PKA-dependent regulation of the CFTR channel.

CFTR contains a large intracellular regulatory (R) domain where multiple PKA phosphorylation sites are located. There are two regions within the R domain that

contain a high proportion of negatively charged amino acids, a.a. 725-733 (NEG1) and a.a. 817-838 (NEG2). It is possible that these two regions could have allosteric or electrostatic interactions with other regions of CFTR and thus affect its chloride channel function. To test the role of NEG1 and NEG2, two deletion mutants, NEG1-CFTR and NEG2-CFTR, were created. The CFTR mutants were transiently expressed in HEK 293 cells, and their single channel functions were studied using the bilayer reconstitution system. Western blots indicate that both NEG1-CFTR and NEG2-CFTR process normally and traffic to the plasma membrane of HEK 293 cells. Both mutants form functional chloride channels in the bilayer membrane, with single channel conductances similar to the wt-CFTR channel. Like wt-CFTR, opening of NEG1-CFTR requires absolutely PKA phosphorylation and ATP binding/hydrolysis. In contrast to wt-CFTR, opening of NEG2-CFTR does not require PKA phosphorylation. Thus, deletion of NEG2, but not NEG1, alters PKA-dependent regulation of the CFTR chloride channel. Our data suggest that NEG2 could form a 'putative gating particle' of the CFTR channel possibly through electrostatic and/or allosteric interactions with other domains of CFTR.

Example 2: *Δ NEG1- and Δ NEG2-CFTR are glycosylated.*

The R domain of CFTR contains two negatively charged regions, amino acids 725-733 (NEG1) and amino acids 817-838 (NEG2), that reside in close proximity to two PKA phosphorylation sites, S737 and S813, used in vivo (Figure 1A) (Cheng, et al. 1991). NEG2 is predicted to form an amphipathic (-helical structure with a negatively charged face (Figure 1B) (Geourjon and Deleage, 1995, Rost and Sander, 1993, Rost and Sander, 1994). Three mutations (E822K, E826K, D836Y), two of

which were clearly obtained from patients with CF (E822K and D836Y), have been identified within the NEG2 region that result in the removal of negative charges (www.genet.sickkids.on.ca). The E822K CFTR channel has a low open probability relative to wt-CFTR (wild type-CFTR), but the E826K CFTR channel has single channel properties similar to wt-CFTR (Vankeerberghen et al., 1998). The presence of these disease-causing mutations suggests the potential importance of the NEG2 region. To investigate the roles of NEG1 and NEG2 in CFTR function, these regions were deleted from CFTR using mutagenesis and subcloning. The ΔNEG1- and ΔNEG2-CFTR proteins were transiently expressed in human embryonic kidney 293 cells. Membrane vesicles containing the CFTR proteins were isolated and subjected to SDS-PAGE. Like wt-CFTR, both ΔNEG1- and ΔNEG2-CFTR are present both in the core glycosylated (band B) and the fully glycosylated form (band C) (Figure 1C).

Example 3: *The open probability of the ΔNEG2-CFTR chloride channel is much less than that of wild type but is independent PKA, although it contains all PKA phosphorylation sites.*

Single channel measurements indicate that the ΔNEG1-CFTR channel is similar to wt-CFTR in its PKA dependence. No chloride channels are observed in the absence of PKA (Figure 2A) and the open probability in the presence of PKA and ATP is similar to wt-CFTR. In contrast, the ΔNEG2-CFTR channel opens without PKA (Figure 2A). The constitutive activity of the ΔNEG2-CFTR channel is unlikely to be due to the endogenous phosphorylation of the ΔNEG2-CFTR protein, since protein phosphatase 2A, which decreases activity of the wt-CFTR opened by PKA and ATP (Ma et al., 1997), has no effect on the ΔNEG2-CFTR channel (n=4). Moreover, addition of PKA up to 200 units/ml, four times the concentration required to fully

activate wt-CFTR (Ma et al., 1997), does not increase the open probability of the channel (Figure 2B). ΔNEG2-CFTR has conductance properties similar to wild type (Tao et al., 1996). However, the open probability of the ΔNEG2-CFTR chloride channel is much less than that of wild type and cannot be increased by PKA (P_o= 0.035 (0.012 and P_o= 0.026 (0.013 without and with PKA respectively, n=5). While NEG2 may represent an inhibitory region, removal of these amino acids does not result in a fully activated channel. The failure of the ΔNEG2-CFTR channel to respond to PKA does not result from inability of the channel to be phosphorylated, for an in vitro assay using (-^{32}P-ATP showed comparable phosphorylation of wt-CFTR and ΔNEG2-CFTR (Figure 1C). Thus, it appears that removal of NEG2 from CFTR completely eliminates the PKA dependence of the chloride channel, although the ΔNEG2-CFTR channel still contains all ten PKA sites and can be phosphorylated.

Example 4: *NEG2 polypeptide stimulates both wild-type and Δ NEG2 CFTR proteins at concentrations greater than 0.44 μM.*

To test whether the NEG2 region is responsible for both stimulatory and inhibitory interactions between the R domain and other domains, synthetic NEG2, a 22 amino acid peptide, was added to the cis-intracellular side of single CFTR channels captured in the planar lipid bilayer (Figure 3). The diary plot of open probability as a function of time shows the activity of a single wt-CFTR channel during the course of the experiment (Figure 3A). After peptide addition, there are periods of intense stimulation that last 4 to 8 minutes. These stimulatory periods are followed by either a return to the basal level of activity before peptide addition, or by an almost complete inhibition of the channel, where only a flickery 3 pS conductance is observed. During stimulation, the open probability more than doubles and more transitions are observed

between the open and closed states (Figure 3B). The stimulatory response was observed in 6 of 7 experiments at concentrations ≥0.44 μM (the remaining channel was inhibited upon peptide addition (4.4 μM) and no stimulation was seen). Profound inhibition was observed in three channels at concentrations ≥ 4.4 μM. When the NEG2 peptide was added to the intracellular side of the ΔNEG2-CFTR channel, which lacks its own endogenous NEG2 sequence, a similar stimulatory response was observed (Figure 3C).

Example 5: *The NEG2 peptide decreases the closed time of the wild-type CFTR protein.*

In order to understand the mechanism responsible for the increase in open probability, the gating kinetics of wt-CFTR without peptide and during stimulation by synthetic NEG2 were analyzed. The open time distributions of the wt-CFTR did not change during peptide stimulation, as both control (without NEG2 peptide) and peptide-stimulated channels had an open lifetime of approximately 120 ms (Figure 4A). Thus, the increase in the open probability is not due to a change in the closing rate of the channel. However, the closed time distribution for the stimulated channel is clearly shifted to the left compared to the control channel (Figure 4B). There are three components to the closed state, a fast (τ_{c1}), an intermediate (τ_{c2}), and a long (τ_{c3}) closed component. The fast closed component is probably due to closings within a burst (Carson et al., 1995). Therefore, to identify better the closed times between bursts, a delimiter of τ_c = 40 ms was set at the nadir between the fast and intermediate closed times (illustrated by the arrow in Figure 4B) to generate the closed-burst duration histograms. As shown in Figure 4C, following peptide stimulation, the intermediate closed time was reduced from 459 ms to 105 ms, whereas the long closed time

remained relatively unchanged. Thus, the interaction of NEG2 with CFTR increased the intermediate-opening rate of the channel. This increase in opening rate is similar to that observed when the phosphorylated R domain protein (amino acids 645-834) was added to CFTR- ΔR/S660A in excised, inside-out patches (Winter and Welsh, 1997). Additionally, modification of C832, which resides within NEG2, by N-ethylmaleimide (NEM) results in irreversible stimulation of PKA-phosphorylated CFTR chloride channel activity (Cotten and Welsh, 1997), further emphasizing the importance of NEG2 in CFTR regulation.

These data, taken together, show that the NEG2 region confers both stimulatory and inhibitory functions of the R domain on the CFTR channel. When this region is deleted from CFTR, the resultant channel opens without PKA (loss of inhibitory function), but it never achieves open probability comparable to wild type even when phosphorylated with PKA (loss of stimulatory function). This same sequence, expressed as a peptide, results in stimulation of channel openings at lower concentrations and profound inhibition of channel activity at higher concentrations, when added to the intracellular side of CFTR channels. It seems likely that this sequence interacts with CFTR at different sites on the nucleotide binding domains to either stimulate or inhibit channel openings. Phosphorylation of the R domain, in this model, changes its conformation and thus presents the NEG2 sequence better to the stimulatory than the inhibitory site. A current model for channel opening is that phosphorylated channels open in response to ATP binding and hydrolysis at the first nucleotide binding fold (NBF1) (Gadsby and Nairn, 1994, Ma and Davis, 1998). Since stimulation by NEG2 occurs by increasing channel openings, a likely site of stimulation is NBF1, though other models are possible.

METHODS USED IN EXAMPLES 1-5

Subcloning of CFTR gene

The wt, ΔNEG1-, and ΔNEG2-CFTR cDNAs were subcloned into an Epstein-Barr virus-based episomal eukaryotic expression vector, pCEP4 (Invitrogen, San Diego, CA), between the Nhe1 and Xho1 restriction sites. The ΔNEG1 and ΔNEG2 deletion mutants were created using the pALTER mutagenesis system and shuttled from pALTER into pCEP4 by substituting the corresponding fragment in pCEP4 wt-CFTR with the mutant fragment between the Xho1 and BstZ171 restriction sites. The ΔNEG1-CFTR cDNA has 27 bases deleted (amino acids 725-733). The ΔNEG2-CFTR cDNA has 66 bases deleted (amino acids 817-838).

Expression of CFTR in HEK 293 cells

A human embryonic kidney cell line (293-EBNA HEK; Invitrogen) was used for transfection and expression of the CFTR proteins (Ma et al., 1997, Ma et al., 1996, Xie et al., 1995). The HEK-293 cell line contains a pCMV-EBNA vector, which constitutively expresses the Epstein-Barr virus nuclear antigen-1 (EBNA-1) gene product and increases the transfection efficiency of Epstein-Barr virus-based vectors. The cells were maintained in Dulbecco's Modified Eagle Medium with 10% FBS and 1% L-glutamine. Geneticin (G418, 250 (g/ml) was added to the cell culture medium to maintain selection of the cells containing the pCMV-EBNA vector. Lipofectamine reagent (Life Technologies, Inc) in Optimem media (serum-free) was used to transfect the HEK-293 cells with pCEP4(wt), pCEP4(ΔNEG1), or pCEP4(ΔNEG2). After 5 hours, serum was added to the media (10% final serum concentration). Twenty-four hours after transfection, the transfection media was replaced with fresh media. The

cells were harvested two days after transfection and microsomal membrane vesicles were prepared for single channel measurements in the lipid bilayer reconstitution system.

Vesicle preparation from transfected HEK 293 cells

HEK-293 cells transfected with pCEP4(CFTR) were harvested and homogenized using a combination of hypotonic lysis and Dounce homogenization in the presence of protease inhibitors (Ma et al., 1997, Ma et al., 1996, Xie et al., 1995). Microsomes were collected by centrifugation of postnuclear supernatant (4500 x g, 15 min) at 100,000 x g for 20 min and resuspended in a buffer containing 250 mM sucrose, 10 mM HEPES, pH 7.2. The membrane vesicles were stored at -75°C until use.

In vitro phosphorylation of CFTR proteins

CFTR proteins isolated in membrane vesicles were bound to protein G agarose using a mouse monoclonal anti-human CFTR antibody (Genzyme). The protein G agarose was washed, and (-^{32}P-ATP (10 (Ci) and protein kinase A (~10 units/50(l) was added. Samples were incubated at 30(C for one hour during phosphorylation. Excess (γ-^{32}P-ATP was removed, and SDS-PAGE sample buffer (200 mM Tris-Cl, pH 6.7, 9% SDS, 6% beta-mercaptoethanol, 15% glycerol, and 0.01% bromophenol blue) was added to denature CFTR and release it from the protein G agarose. The samples were subjected to electrophoresis on a 5% SDS-polyacrylamide gel, transferred to a polyvinylidene difluoride membrane, and exposed to film.

Preparation of NEG2 peptides

The 22 amino acid peptide corresponding to NEG2 was custom made by Quality Controlled Biochemicals, Inc. The peptide was resuspended in water to a concentration of 1 mg/ml and pH was adjusted to a physiological range (7.2-7.4) using KOH and HCl. The space filling model of the NEG2 peptide was generated, based on secondary structure predictions (Geourjon and Deleage, 1995, Rost and Sander, 1993, Rost and Sander, 1994), using the Insight II program from Molecular Simulations Incorporated.

Reconstitution of CFTR channels in lipid bilayer membranes

Lipid bilayer membranes were formed across an aperture of ~200 (m diameter with a mixture of phosphatidylethanolamine:phosphatidylserine:cholesterol in a ratio of 5:5:1. The lipids were dissolved in decane at a concentration of 33 mg/ml. The recording solutions contained: cis (intracellular), 200 mM CsCl, 1 mM $MgCl_2$, 2 mM ATP, and 10 mM HEPES-Tris (pH 7.4); trans (extracellular), 50 mM CsCl, 10 mM HEPES-Tris (pH 7.4). Vesicles (1-4 (l) containing either wild-type, ΔNEG1-, or ΔNEG2-CFTR were added to the cis solution. The PKA catalytic subunit was present at a concentration of 50 units/ml in the cis solution unless noted otherwise. Single channel currents were recorded with an Axopatch 200A patch clamp unit (Axon Instruments). The currents were sampled at 1-2.5 ms/point. Single channel data analyses were performed with pClamp and TIPS softwares.

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CLAIMS:

1. An isolated polypeptide comprising a portion of CFTR (cystic fibrosis transmembrane conductance regulator) protein of between 10 and 100 amino acids, said portion comprising 18 amino acids as shown in SEQ ID NO: 1.

2. The polypeptide of claim 1 which comprises 22 amino acids as shown in SEQ ID NO: 2.

3. The polypeptide of claim 1 wherein the polypeptide is fused to a membrane-penetrating peptide.

4. The polypeptide of claim 2 wherein the polypeptide is fused to a membrane-penetrating peptide.

5. The polypeptide of claim 3 wherein the membrane-penetrating peptide is selected from the group consisting of: VP-22 (SEQ ID NO: 3), (SEQ ID NO: 4), and (SEQ ID NO: 5).

6. The polypeptide of claim 4 wherein the membrane-penetrating peptide is selected from the group consisting of: VP-22 (SEQ ID NO: 3), (SEQ ID NO: 4), and (SEQ ID NO: 5).

7. The polypeptide of claim 1 which is free of phosphorylation.

8. A method of activating a CFTR protein comprising:

applying a polypeptide to a CFTR protein which forms a cAMP regulated chloride channel, said polypeptide comprising a portion of CFTR protein of between about 10 and 100 amino acids, said portion comprising 18 amino acids as shown in SEQ ID NO: 1, whereby the open probability of the channel formed by the CFTR increases by at least 25%.

9. The method of claim 8 wherein the open probability of the channel formed by the CFTR increases by at least 50%.

10. The method of claim 8 wherein the open probability of the channel formed by the CFTR increases by at least 75%.

11. The method of claim 8 wherein the open probability of the channel formed by the CFTR increases by at least 100%.

12. The method of claim 8 wherein the open probability of the channel formed by the CFTR increases by at least 125%.

13. The method of claim 8 wherein the open probability of the channel formed by the CFTR increases by at least 150%.

14. The method of claim 8 wherein the open probability of the channel formed by the CFTR increases by at least 200%.

15. The method of claim 8 wherein the CFTR protein is a mutant which reaches a cell's plasma membrane but fails to undergo full activation.

16. The method of claim 15 wherein the CFTR protein is listed at http://www.genet.sickkids.on.ca/cftr-cgi-bin/fulltable.

17. The method of claim 8 wherein the step of applying is performed by administering an aerosolized polypeptide to a patient with a mutant CFTR protein.

18. The method of claim 8 wherein the CFTR protein is in a lipid bilayer and a change in conductance is measured upon applying the polypeptide.

19. The method of claim 8 wherein the step of applying the polypeptide is accomplished by administering a nucleic acid encoding the polypeptide to a patient who expresses the CFTR protein, whereby the polypeptide is

expressed.

20. The method of claim 19 wherein the nucleic acid is administered as an aerosol to the patient's airways.

21. A method of activating a CFTR protein comprising:

applying a polypeptide to a CFTR protein which forms a cAMP regulated chloride channel, said polypeptide comprising a portion of CFTR protein of between 10 and 100 amino acids, said portion comprising 22 amino acids as shown in SEQ ID NO: 1, whereby the open probability of the channel formed by the CFTR increases by at least 25%.

22. The method of claim 21 wherein the open probability of the channel formed by the CFTR increases by at least 50%.

23. The method of claim 21 wherein the open probability of the channel formed by the CFTR increases by at least 75%.

24. The method of claim 21 wherein the open probability of the channel formed by the CFTR increases by at least 100%.

25. The method of claim 21 wherein the open probability of the channel formed by the CFTR increases by at least 125%.

26. The method of claim 21 wherein the open probability of the channel formed by the CFTR increases by at least 150%.

27. The method of claim 21 wherein the open probability of the channel formed by the CFTR increases by at least 200%.

28. The method of claim 21 wherein the CFTR protein is a mutant which reaches a cell's plasma membrane but fails to undergo full activation.

29. The method of claim 28 wherein the CFTR protein is listed at http://www.genet.sickkids.on.ca/cftr-cgi-bin/fulltable.

30. The method of claim 21 wherein the step of applying is performed by administering an aerosolized polypeptide to a patient with a mutant CFTR protein.

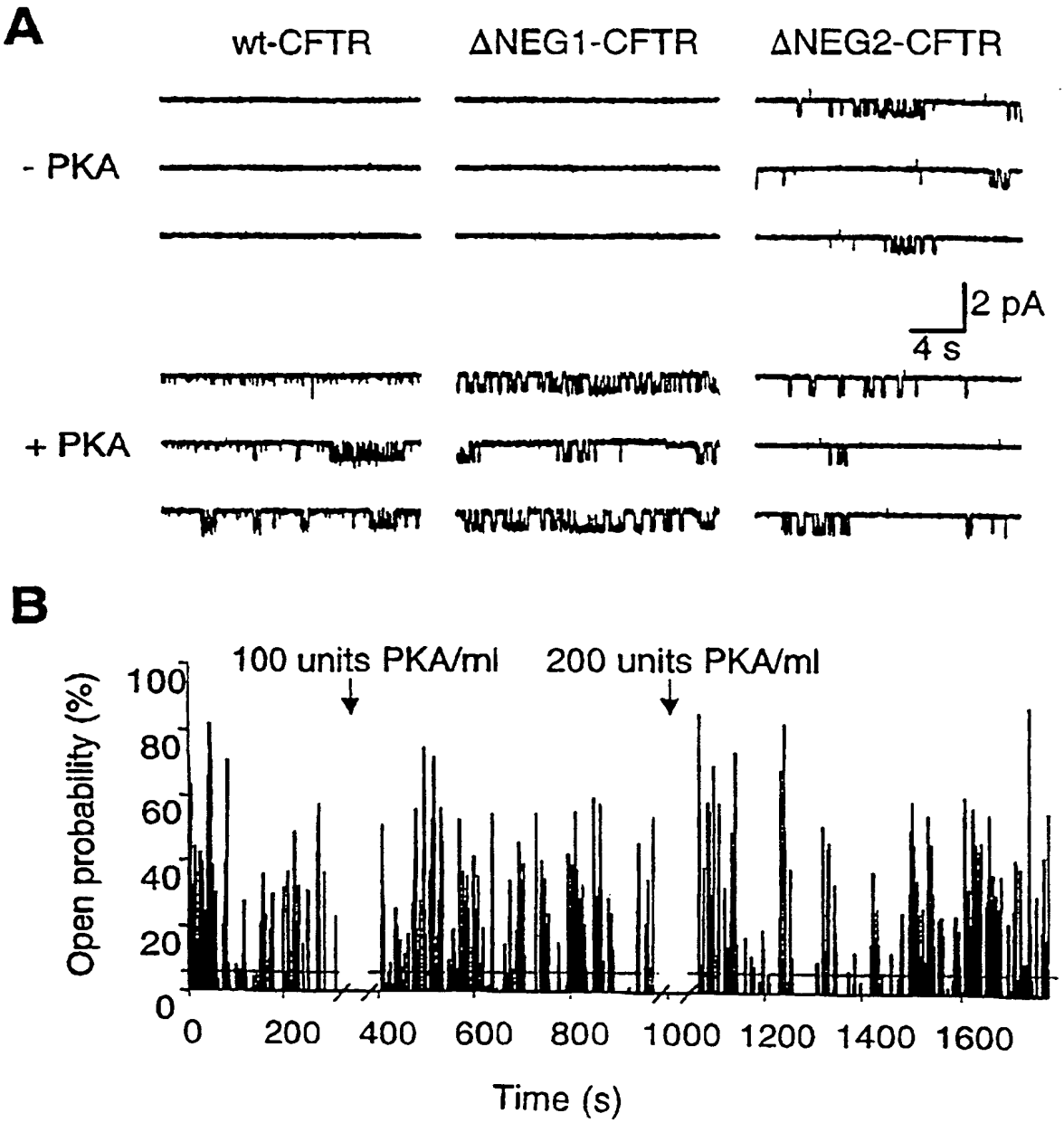
31. The method of claim 21 wherein the CFTR protein is in a lipid bilayer and a change in conductance is measured upon applying the polypeptide.

32. The method of claim 21 wherein the step of applying the polypeptide is accomplished by administering a nucleic acid encoding the polypeptide to a patient who expresses the CFTR protein, whereby the polypeptide is expressed.

33. The method of claim 32 wherein the nucleic acid is administered as an aerosol to the patient's airways.

34. The method of claim 8 or 21 wherein the polypeptide is free of phosphorylation.

Fig. 1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ΔNEG1-CFTR
ΔNEG2-CFTR
- PKA
+ PKA
2 pA
4 s
B
100 units PKA/ml
200 units PKA/ml
Open probability (%)
100
80
60
40
20
0
0
200
400
600
800
1000
1200
1400
1600
Time (s)

Fig. 2

A

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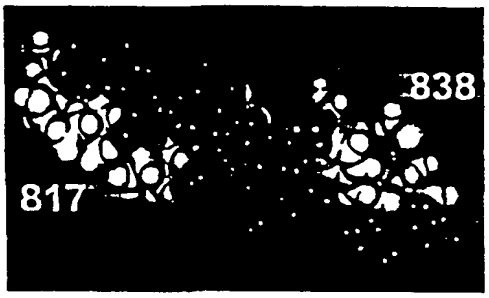
NEG2 817 G L E I S E E I N E E D L K E C F F D D M E 838

B



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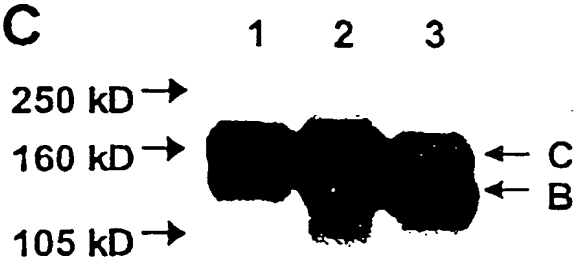

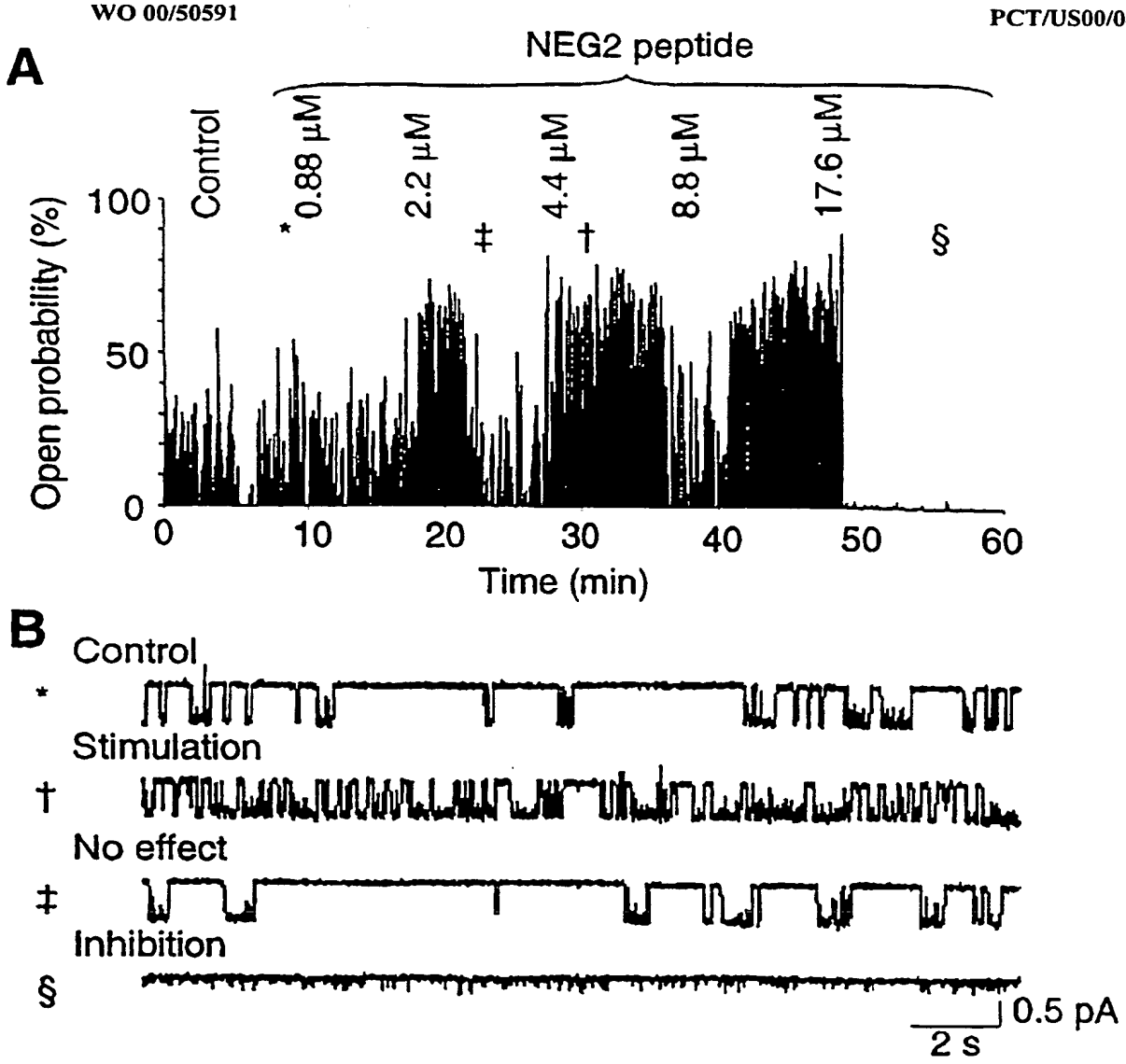
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4.4 µM
8.8 µM
17.6 µM
*
‡
†
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Open probability (%)
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50
0
0
10
20
30
40
50
60
Time (min)
B
Control
*
Stimulation
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‡
Inhibition
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2 s
C
Control
Stimulation
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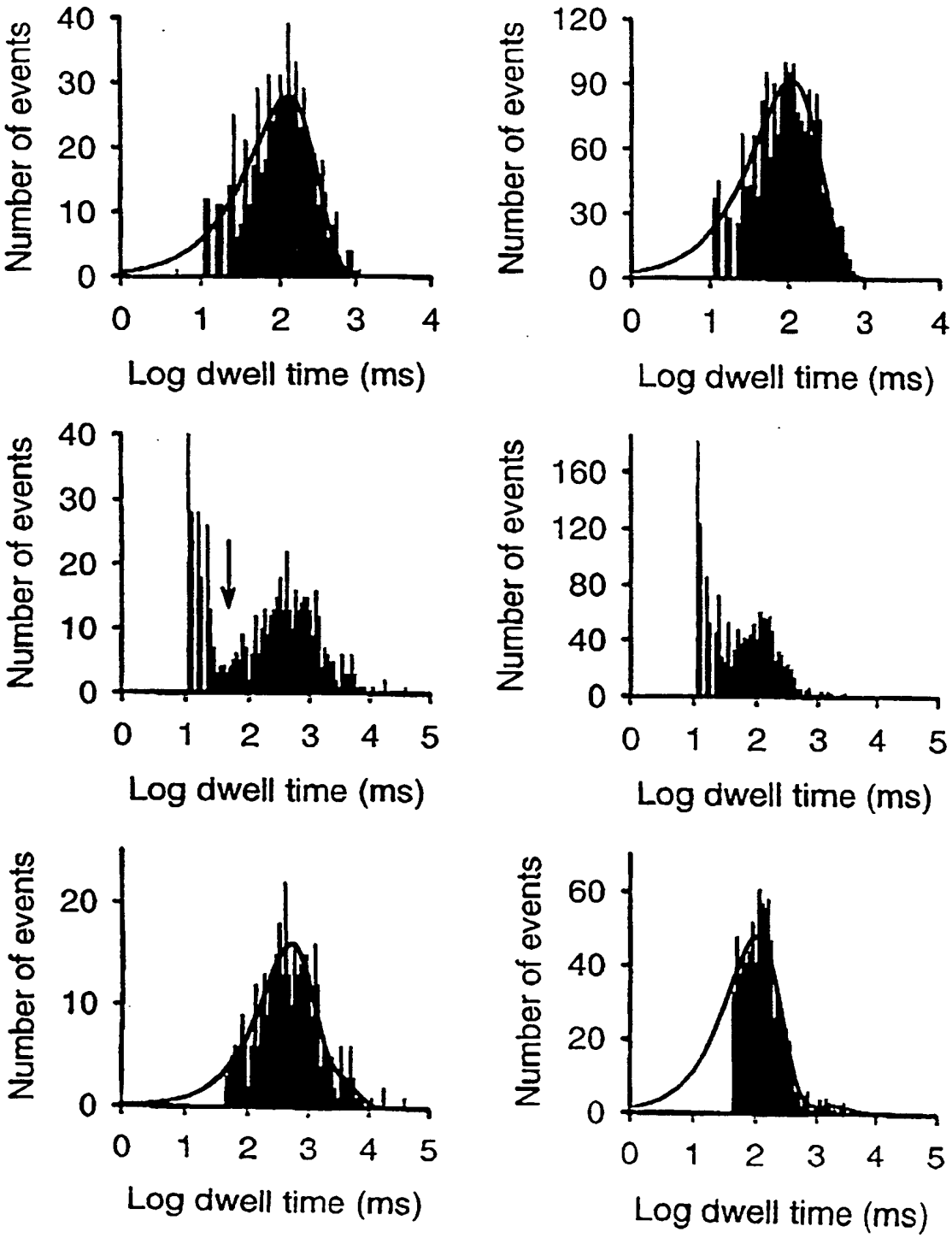
4 s

Fig. 4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SEQUENCE LISTING

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SEQ ID NO: 3 VP-22 (Phelan et al., Nature Biotech 16:440-443, 1998, incorporated by reference herein)

SEQ ID NO: 4 GWTLNSAGYLLGKINLKALAALAKKIL (amide)

SEQ ID NO: 5 RQIKIWFQNRRMKWKK (amide)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/04642

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/62 C07K14/47 A61K38/17 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ADAMS L M ET AL: "Deletion of a negatively charged region (a.a 817-838) from the R domain of CFTR alters PKA-dependent regulation of the CFTR channel." BIOPHYSICAL JOURNAL, vol. 74, no. 2 PART 2, February 1998 (1998-02), page A344 XP000923128 Forty-second Annual Meeting of the Biophysical Society;Kansas City, Missouri, USA; February 22-26, 1998 ISSN: 0006-3495 abstract --- -/--	1,2,7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 July 2000	24/07/2000

Name and mailing address of the ISA	Authorized officer
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